



US007144727B2

(12) **United States Patent**
Akers et al.

(10) **Patent No.:** **US 7,144,727 B2**
(45) **Date of Patent:** **Dec. 5, 2006**

(54) **INTERLINKED CULTURE CHAMBER FOR BIOLOGICALS**

(75) Inventors: **Roger Akers**, Houston, TX (US);
William J. Anderson, Richmond, TX (US); **Stephen S. Navran, Jr.**, Houston, TX (US)

(73) Assignee: **Synthecon, Inc.**, Houston, TX (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

5,194,157 A *	3/1993	Ghezzi et al.	210/646
5,415,770 A *	5/1995	Heskett	210/202
5,449,617 A	9/1995	Falkenberg et al.	
5,576,211 A	11/1996	Falkenberg et al.	
5,637,477 A	6/1997	Spaulding et al.	
5,686,301 A	11/1997	Falkenberg et al.	
5,763,275 A	6/1998	Nagels et al.	
5,998,202 A	12/1999	Schwartz et al.	
6,022,733 A	2/2000	Tam et al.	
6,080,581 A	6/2000	Anderson et al.	
6,255,106 B1 *	7/2001	Marx et al.	435/325
2004/0110273 A1 *	6/2004	Akers et al.	435/283.1

(21) Appl. No.: **10/821,455**

(22) Filed: **Apr. 9, 2004**

(65) **Prior Publication Data**

US 2004/0203140 A1 Oct. 14, 2004

Related U.S. Application Data

(60) Provisional application No. 60/462,722, filed on Apr. 14, 2003.

(51) **Int. Cl.**
C12M 3/06 (2006.01)

(52) **U.S. Cl.** **435/294.1**; 435/297.2;
435/298.2; 210/321.64; 210/257.2; 210/335

(58) **Field of Classification Search** 435/293.1,
435/293.2, 294.1, 297.2, 297.4, 298.2; 210/321.64,
210/321.78, 321.87, 257.2, 335

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,988,623 A	1/1991	Schwartz et al.
5,026,650 A	6/1991	Schwartz et al.
5,104,802 A	4/1992	Rhodes et al.
5,153,131 A	10/1992	Wolf et al.
5,153,133 A	10/1992	Schwartz et al.
5,155,034 A	10/1992	Wolf et al.
5,155,035 A	10/1992	Schwartz et al.

FOREIGN PATENT DOCUMENTS

JP 06134210 A * 5/1994

OTHER PUBLICATIONS

Rai M. et al.; Expression systems for production of heterologous proteins, Current Science, vol. 80, No. 9, May 10, 2001, pp. 1121-1128.

(Continued)

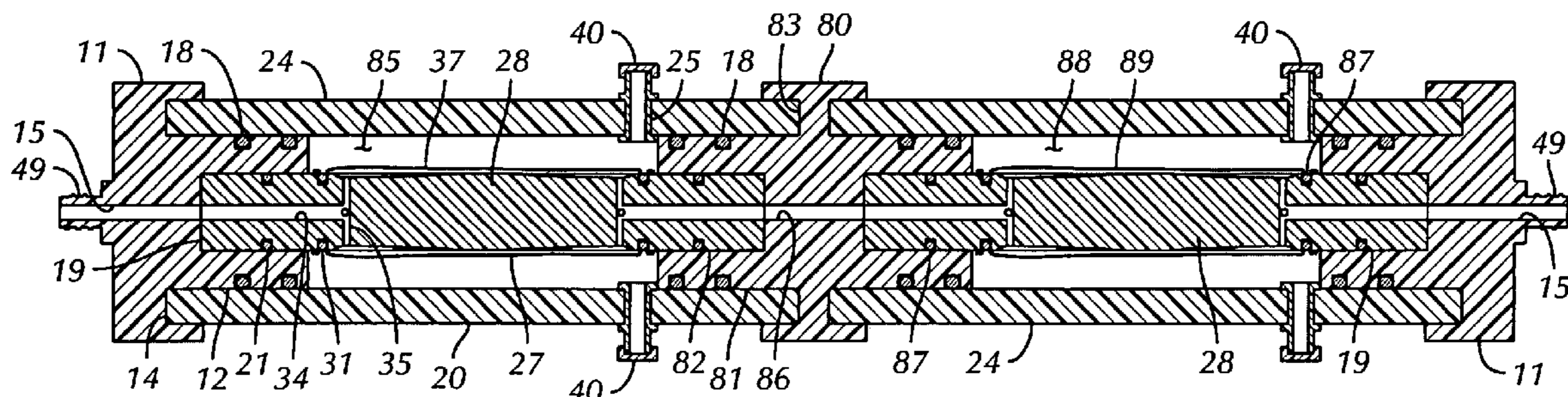
Primary Examiner—William H. Beisner

(74) *Attorney, Agent, or Firm*—Elizabeth R. Hall

(57) **ABSTRACT**

The bioreactor system of the present invention has two fluid-filled culture compartments in which cells, tissues and other biologicals are cultured. The two culture compartments are in fluid communication with each other and each culture compartment is transversed by a filter that prevents the exit of the cells from the culture compartment. Both reusable and disposable culture chambers are described for use as the first and/or second culture compartment.

15 Claims, 12 Drawing Sheets



OTHER PUBLICATIONS

Verma, R. et al.; Antibody engineering: Comparison of bacterial, yeast, insect and mammalian expression systems, Journal of Immunological Methods, 216, 1998, pp. 165-181.

Heraeus Instruments, Inc.; Genetic Engineering News, vol. 14, No. 12, Jun. 15, 1994.

FiberCell Hollow Fiber Cell Culture Systems, Dec. 1, 2004 printout, Internet at bellcoglass.com.

Gerecht-Nir, Sharon, et al. Bioreactor cultivation enhances the efficiency of human embryoid body (hEB) formation and differentiation, Biotechnology and Bioengineering, Early view online Apr. 8, 2004.

* cited by examiner

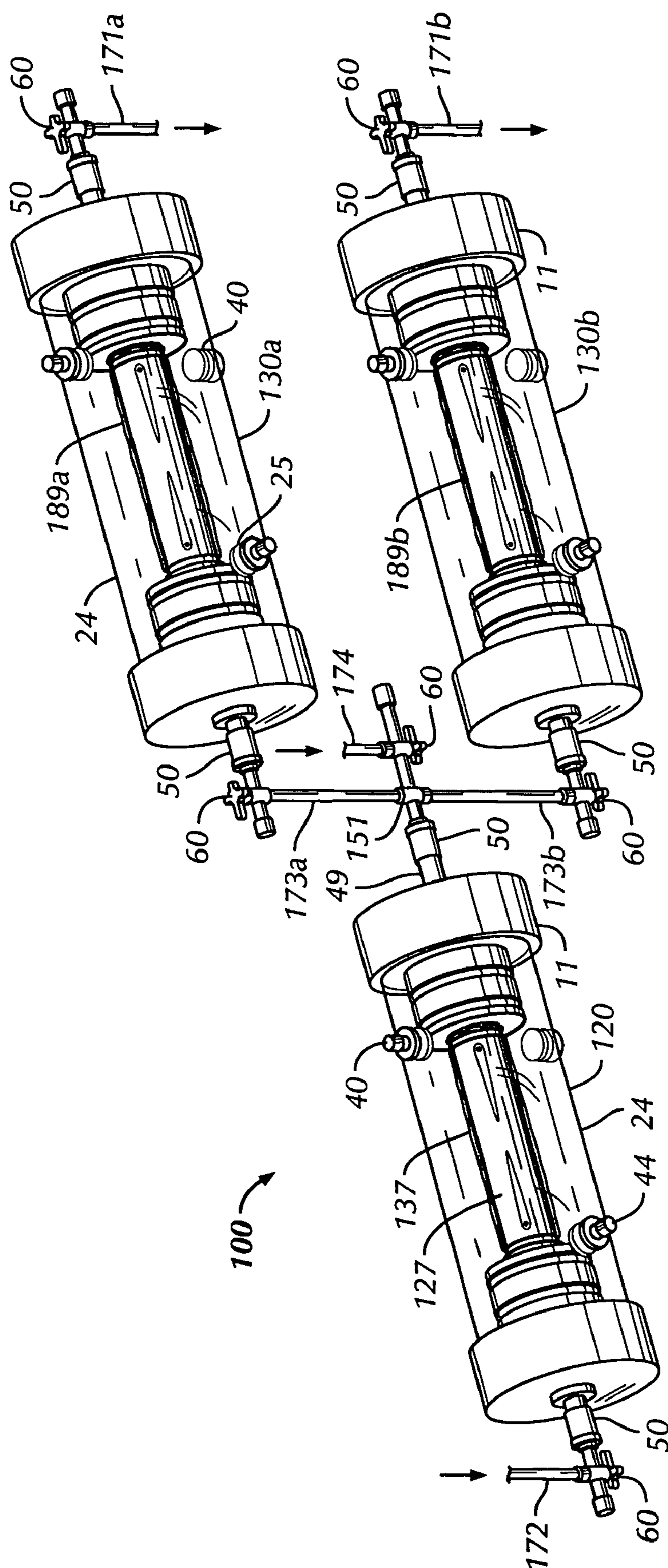


FIG. 1

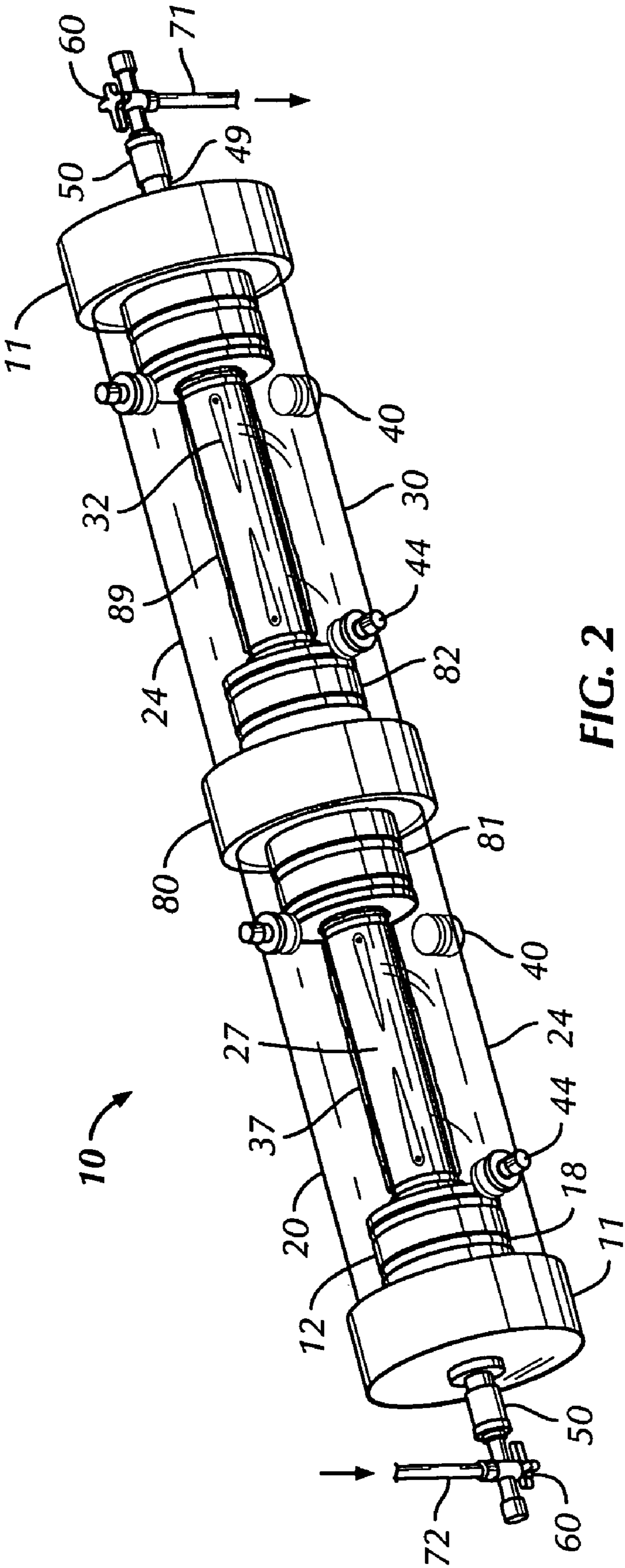


FIG. 2

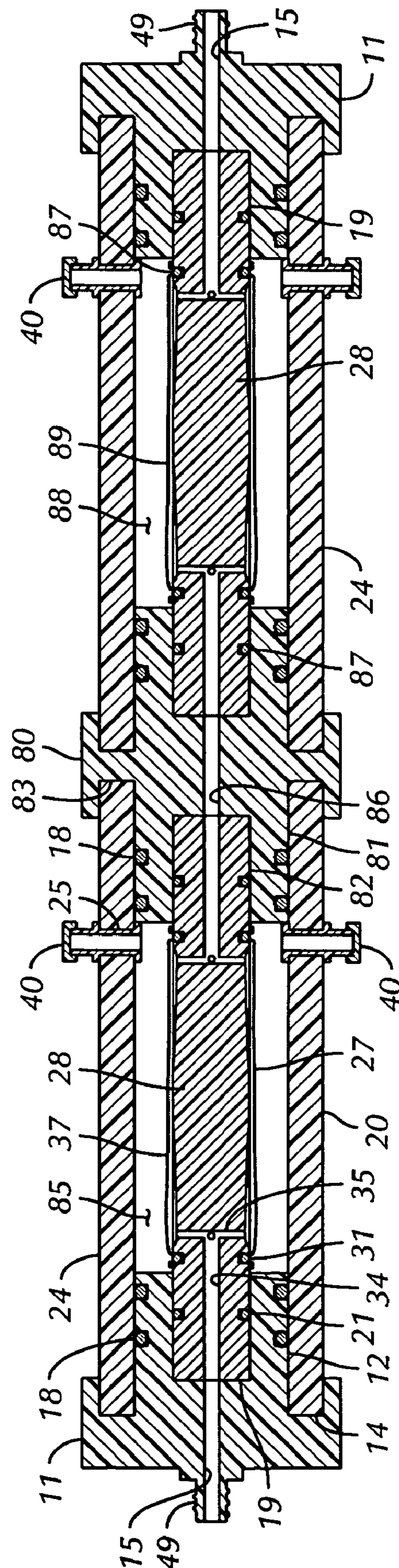
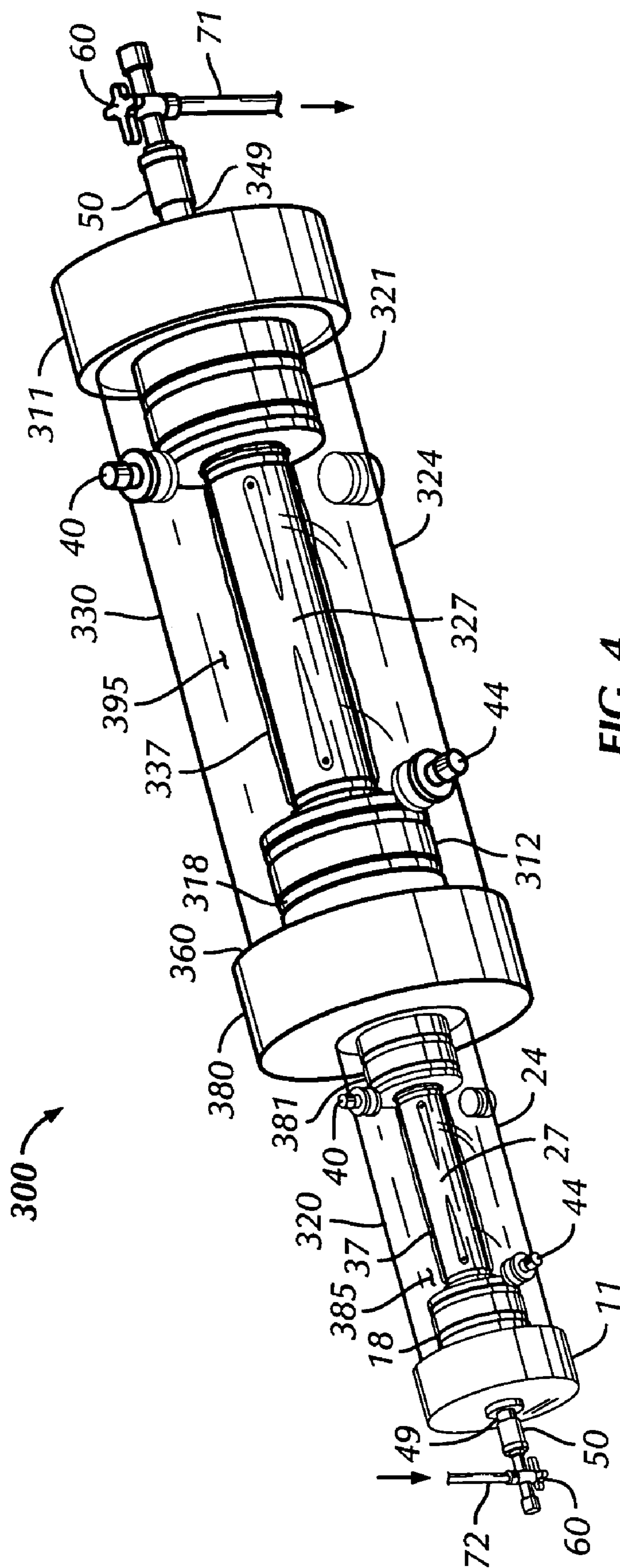


FIG. 3



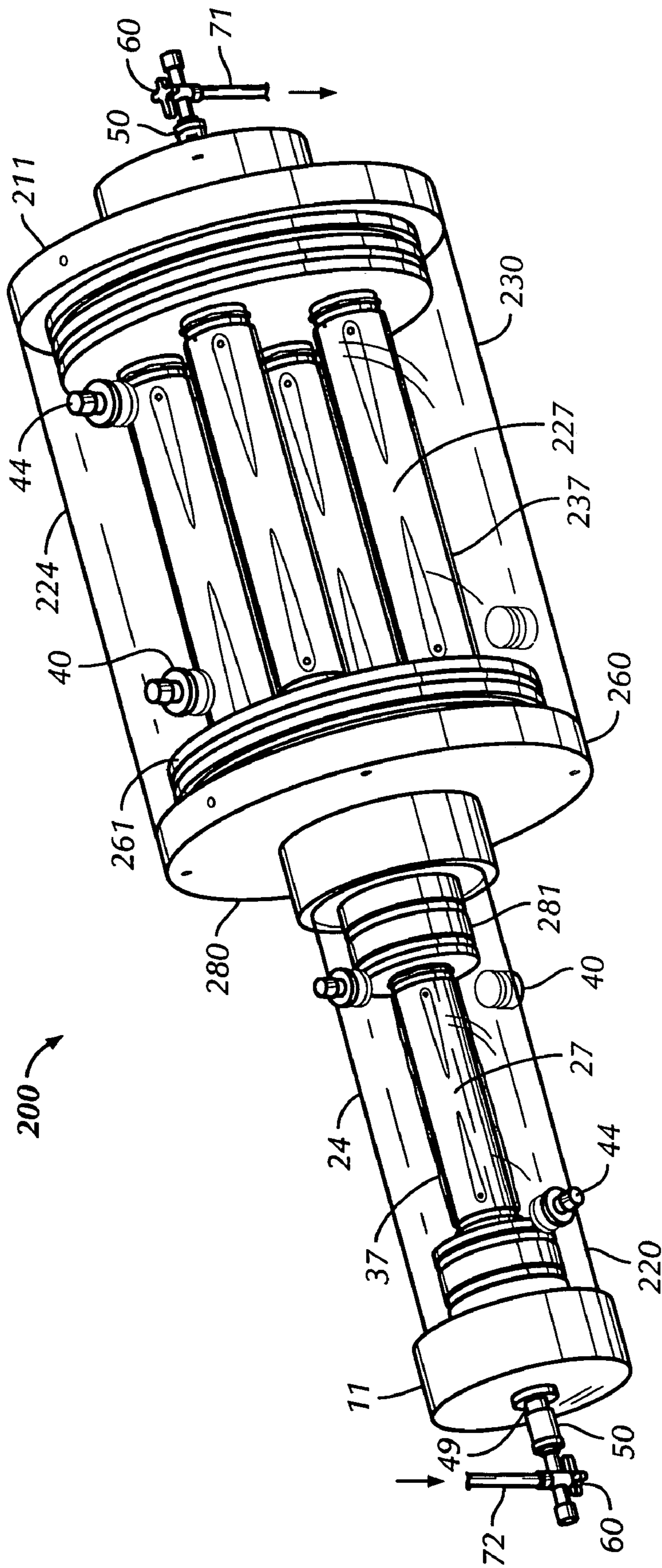


FIG. 5

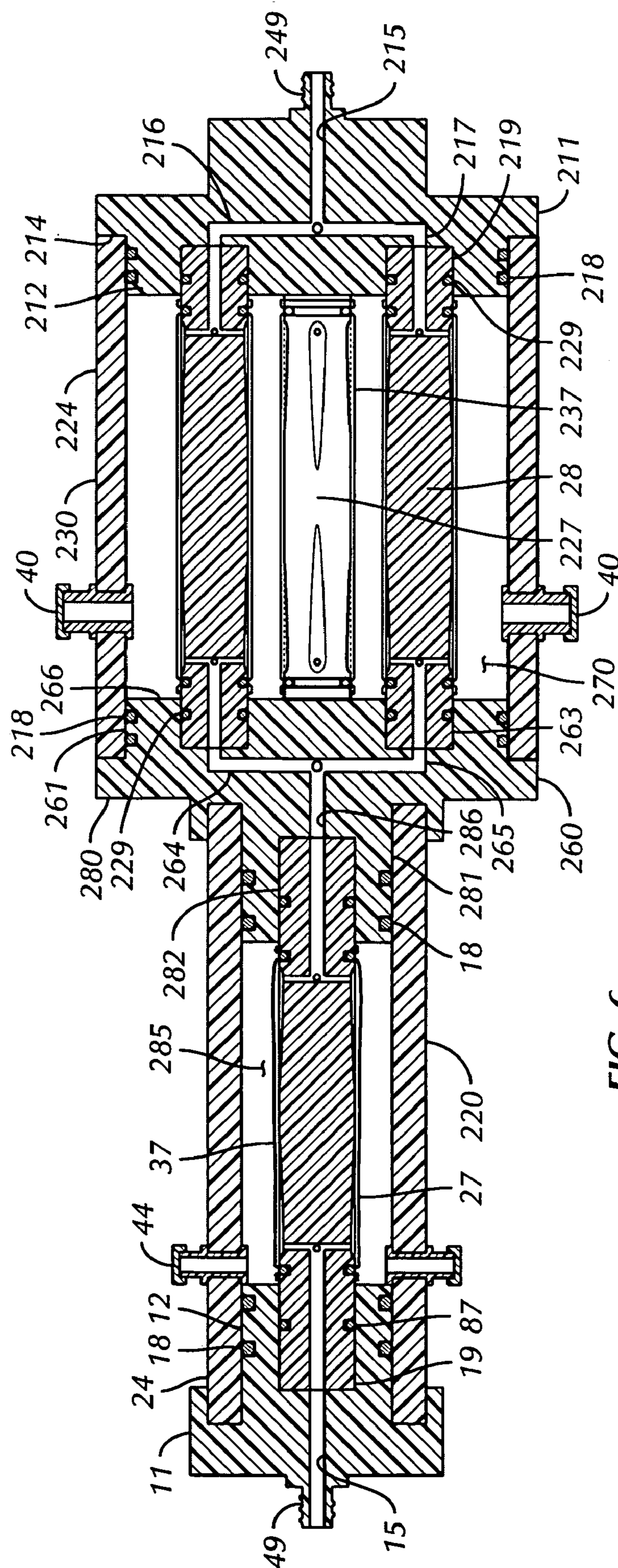


FIG. 6

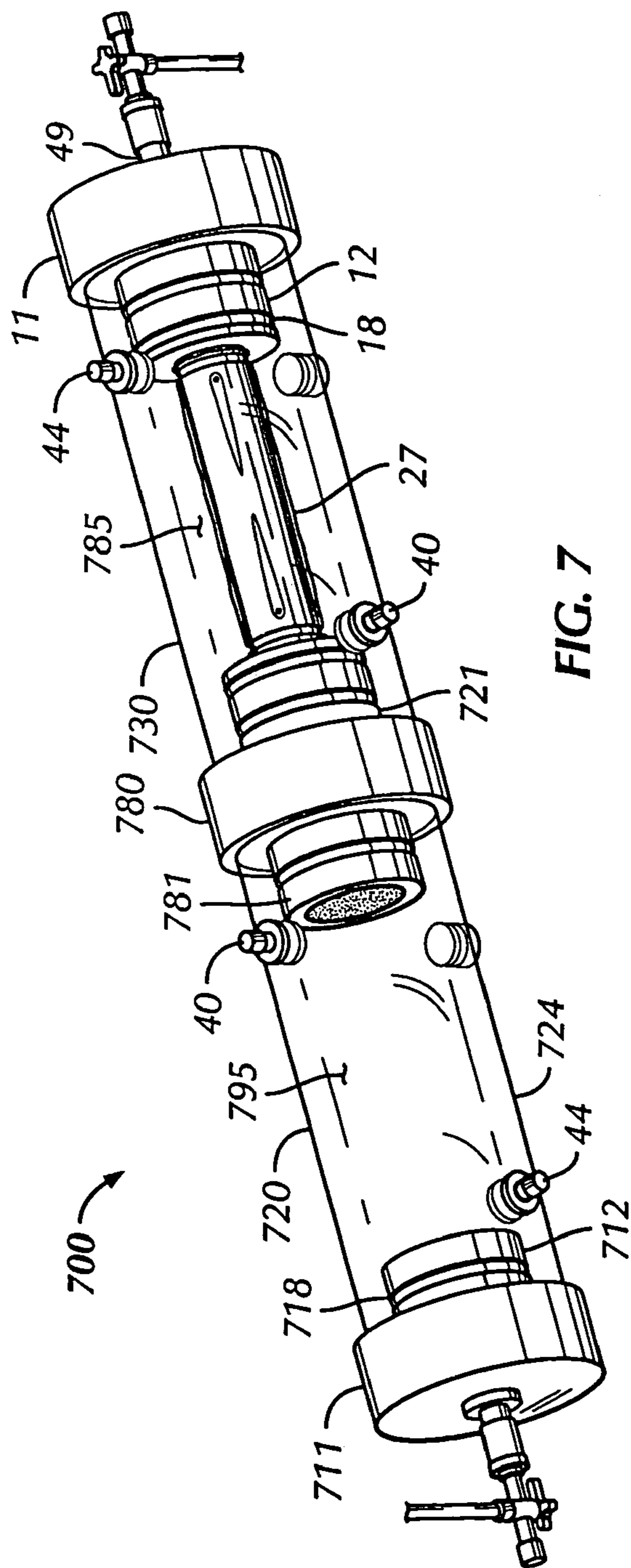


FIG. 7

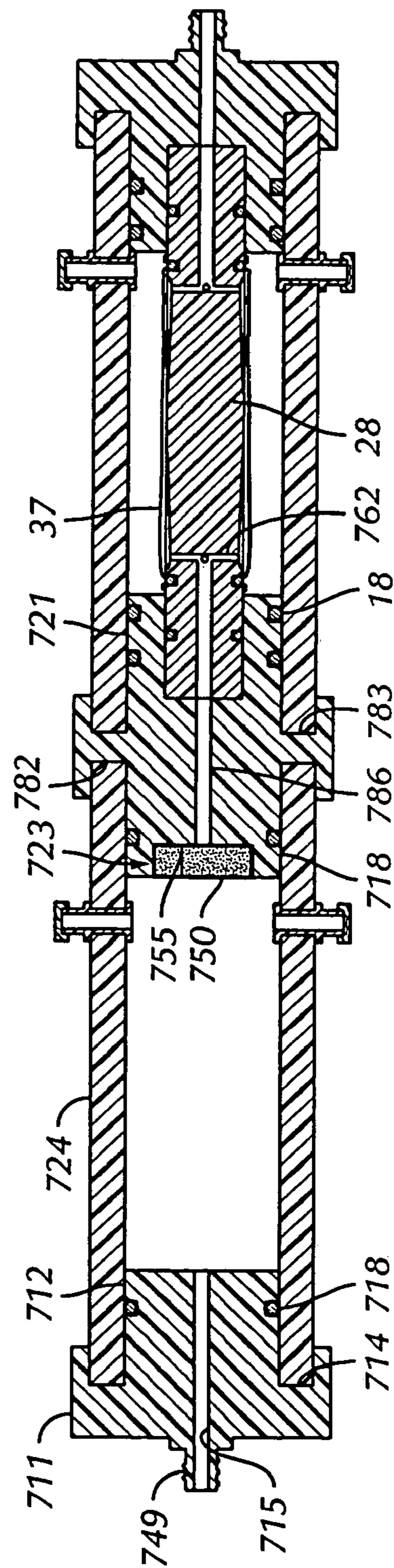


FIG. 8

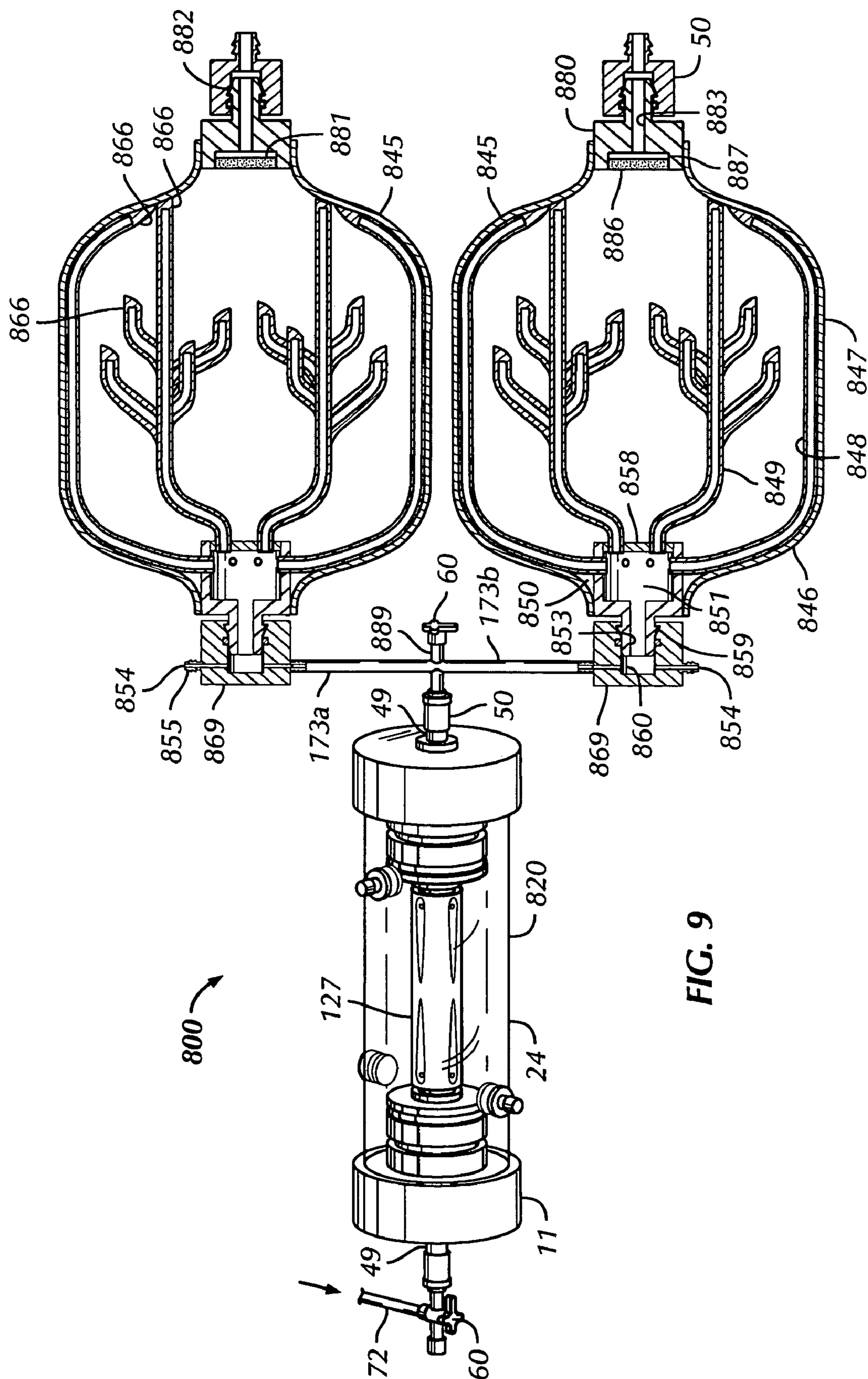


FIG. 9

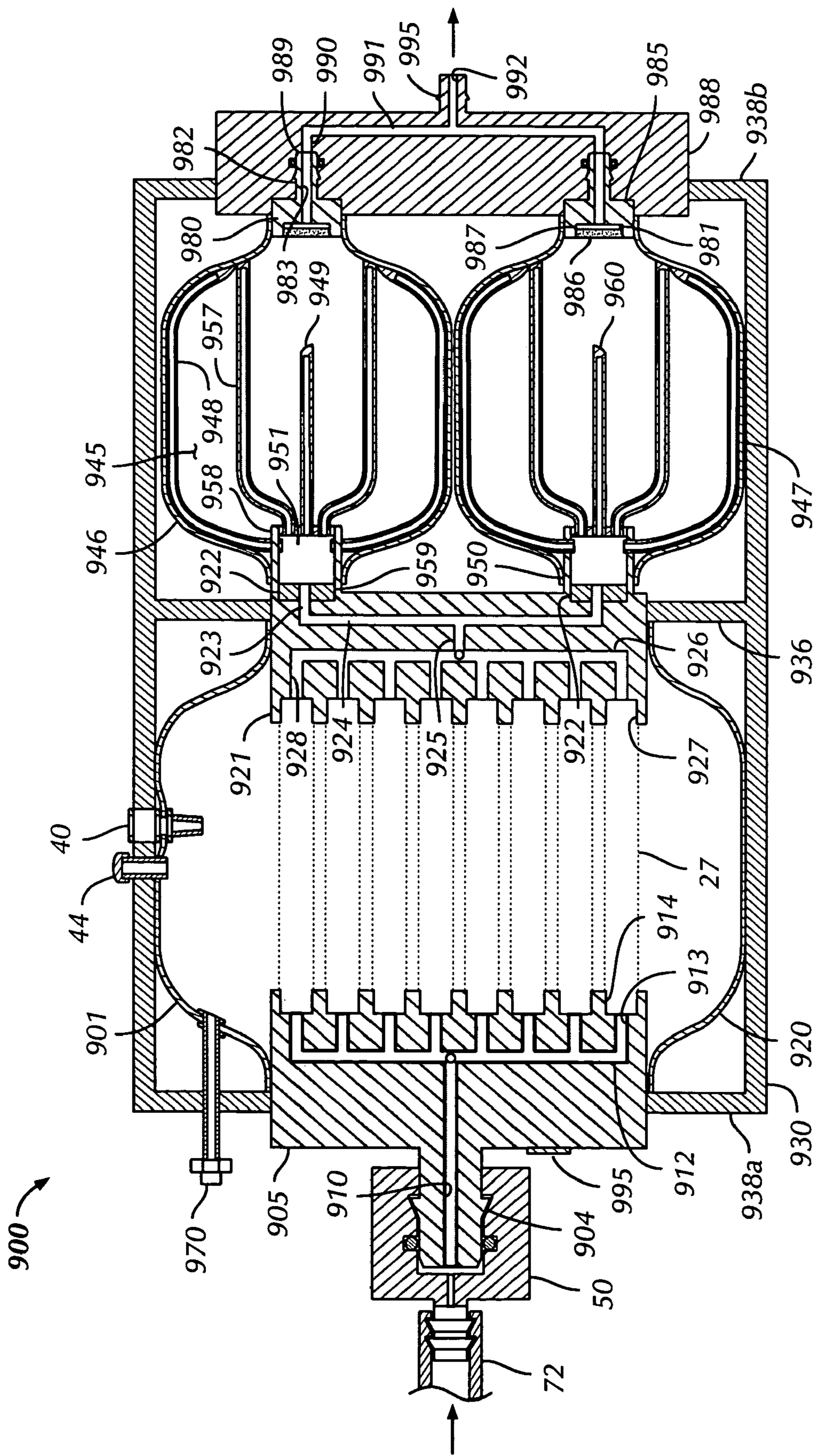


FIG. 10

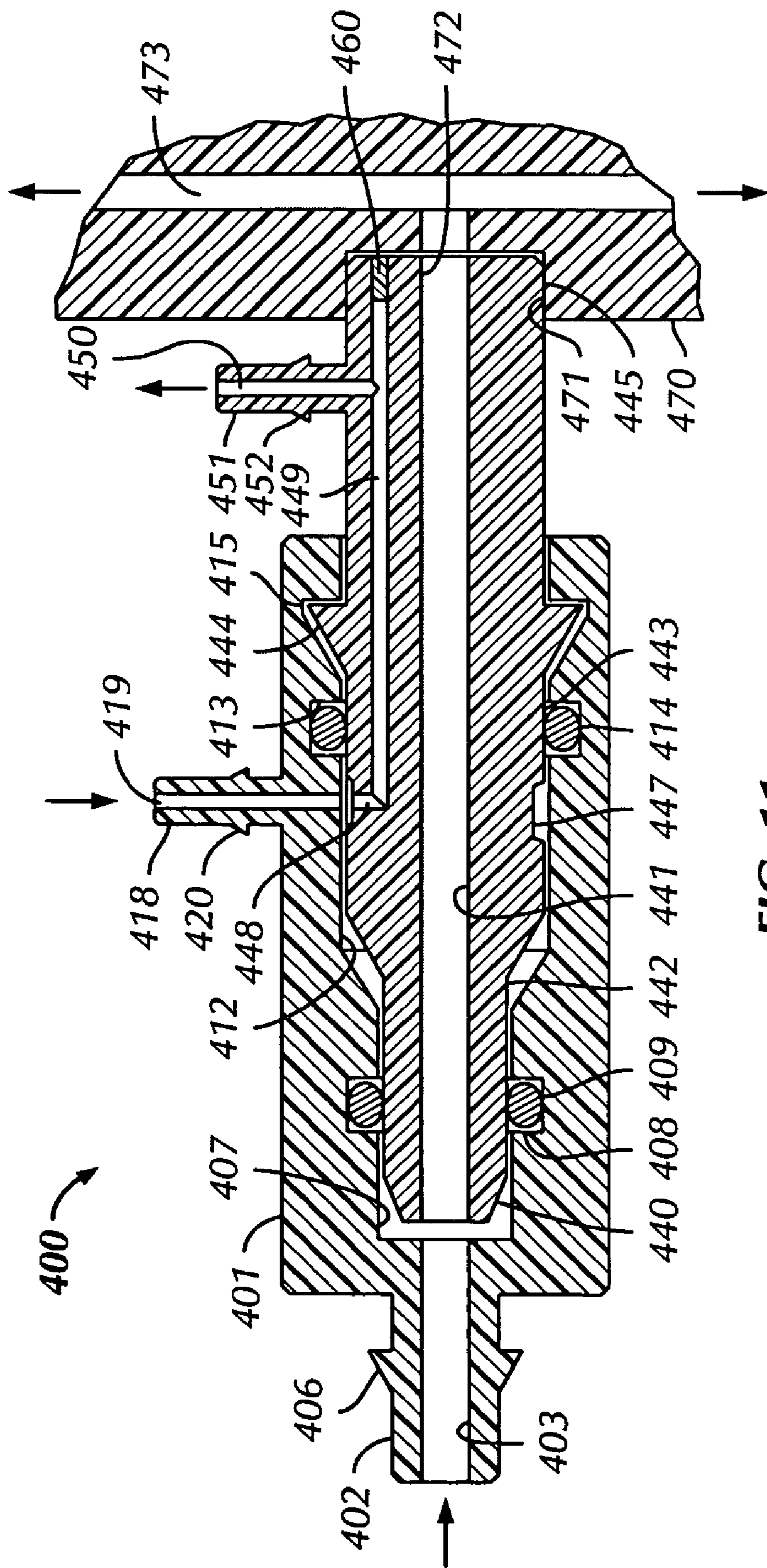


FIG. 11

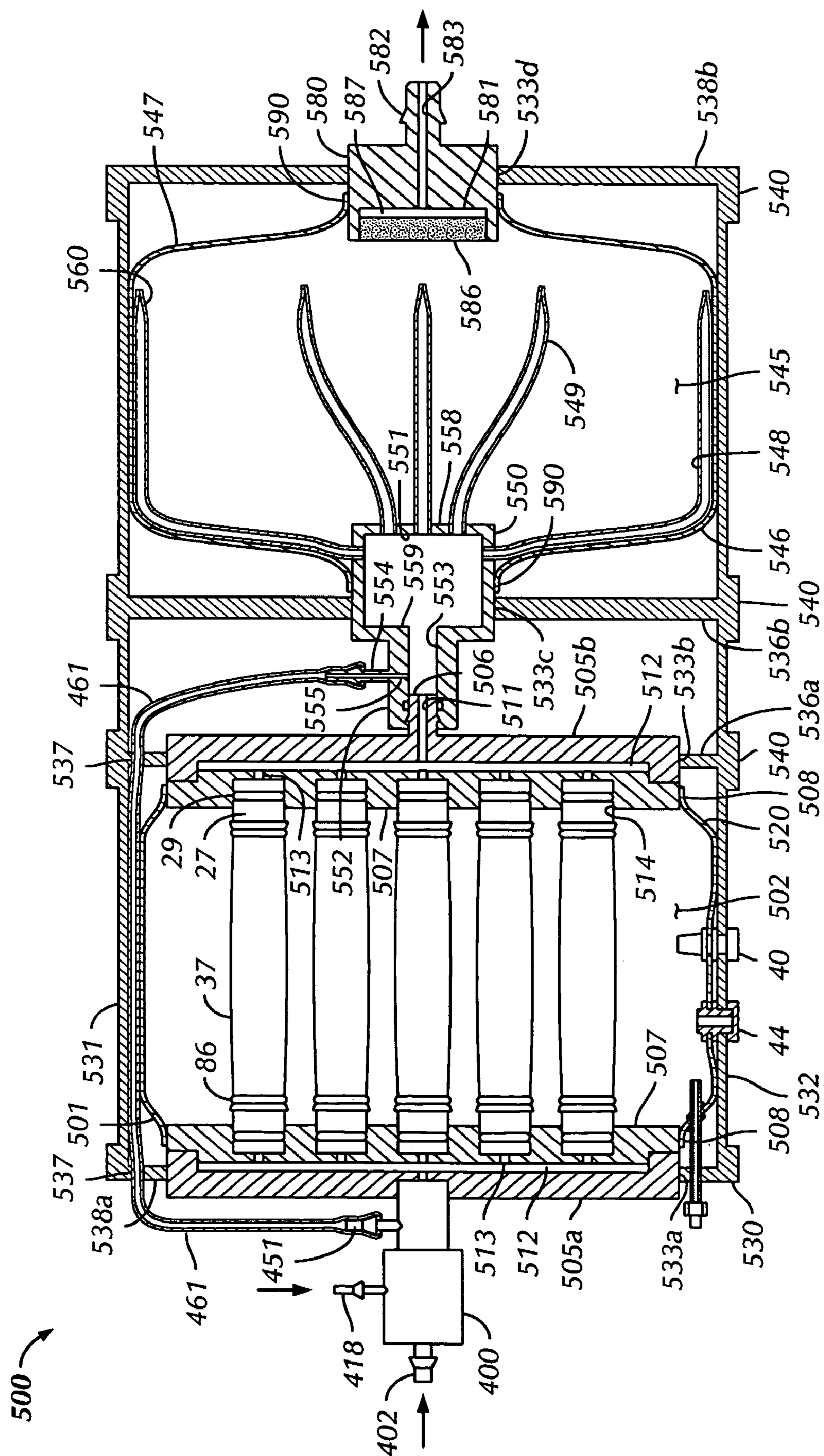


FIG. 12

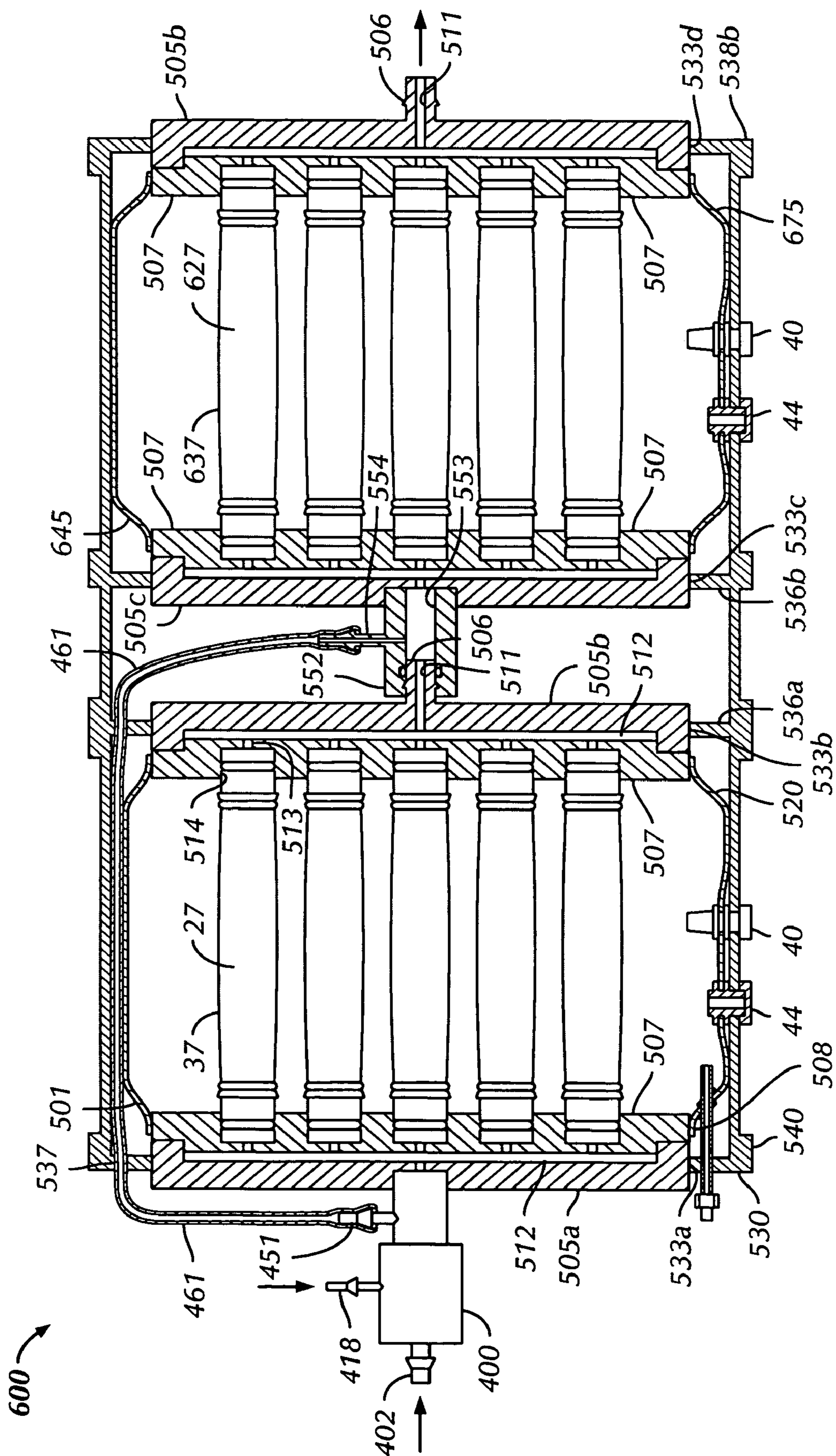


FIG. 13

INTERLINKED CULTURE CHAMBER FOR BIOLOGICALS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to pending U.S. patent application Ser. No. 60/462,722 filed Apr. 14, 2003 by inventors Roger Akers, William Anderson, and Stephen Navran, Jr. and entitled "Interlinked Culture Chambers for Biologicals." The entire text of the above-referenced disclosures is incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a culture chamber for culturing cells, cellular aggregates, particles, tissues and organoids that respond to secretions from other cells, cellular aggregates, particles, tissues and organoids. More particularly, the present invention relates to a culture chamber having a first set of one or more biological culture chambers interconnected with a second set of one or more other biological culture chambers, wherein the secretions of the first set of biological culture chambers is fed into the second set of biological culture chambers.

2. Description of the Related Art

A variety of cell lines, including human embryonic stem (HES) cells require hormones, growth factors or other materials that are secreted from another cell type (commonly referred to as feeder cells). The therapeutic potential of such cells is only beginning to be realized. To keep pace with the ever increasing demand for the potential presented by such cells, new processes and apparatuses are needed that can efficiently provide a first set of cells with the secretions from another set of cells.

One of the major problems in the production of cells and cellular products is the required cleaning, sterilization and validation of the standard stainless steel or glass bioreactors by the customer. Furthermore, none of the currently available culture chambers are designed to have secretions of one culture chamber feed into another culture chamber.

Thus, a need exists for disposable culture chambers having a reduction in the risk of cross contamination and the downtime needed for equipment changeover between production runs.

In addition, a need exists for a simplified, efficient means of interlinking culture chambers such that one can culture one cell and transfer that cell's secretions into the media used to grow another cell type.

SUMMARY OF THE INVENTION

The invention contemplates a culture chamber having a first set of one or more biological culture chambers interconnected with a second set of one or more other biological culture chambers, wherein the secretions of the first set of biological culture chambers is fed into the second set of biological culture chambers. The present invention includes a serially arranged culture chamber system having separate but closely interconnected fluid-filled chambers in which cells, tissues and other biologicals are cultured without intermingling and wherein first, upstream chambers provide growth factors or precursor compounds for the second, downstream chambers.

Each of the upstream culture chambers is either traversed by one or more molecular weight cut-off membranes or

provided with outlet filters that serve to separate the cells of an upstream chamber from the other, downstream flow-streams and chambers.

One aspect of the invention is a culture system comprising: (a) a fluid inlet; (b) a first culture compartment having a tubular housing; (c) a first end piece attached to the fluid inlet on one side and to a first end of the tubular housing on a second side, (d) a second culture compartment coaxial with the first culture compartment, the second culture compartment having a proximal end and a distal end; (e) a fluid connector having a first side mounted on a second end of the tubular housing and a second side mounted on the proximal end of the second culture compartment, the fluid connector having a through bore passing from the first side to the second side of the fluid connector; (f) a connector filter positioned on the first side of the fluid connector to filter a fluid stream passing out of the first culture compartment and into the through bore of the fluid connector; (g) a fluid outlet; (h) a distal end piece mounted on the distal end of the second culture compartment and connected to the fluid outlet; and (i) an outlet filter supported by the distal end piece.

Another aspect of the invention is a culture system comprising: (a) a fluid inlet; (b) a first culture compartment having (i) a tubular sleeve, (ii) a growth compartment within the sleeve, (iii) a first end piece attached to the fluid inlet on one side and to a first end of the tubular housing on a second side, and (iv) a membrane carrier assembly transversing the growth compartment comprising a support cylinder, a molecular weight cut-off membrane secured to an exterior surface of the support cylinder, and a chamber between the exterior surface of the cylinder and an interior surface of the membrane, the chamber in fluid communication with the fluid inlet and the growth compartment; (c) a second culture compartment coaxial with the first culture compartment, the second culture compartment having a proximal end and a distal end; (d) a fluid connector having a first side mounted on a second end of the tubular sleeve and a second side mounted on the proximal end of the second culture compartment, the fluid connector having a through bore passing from the first side to the second side of the fluid connector wherein the through bore is in fluid communication with the chamber of the membrane carrier assembly and the interior of the second culture compartment; (e) a fluid outlet; (f) a distal end piece mounted on the distal end of the second culture compartment and connected to the fluid outlet; and (g) an outlet filter supported by the distal end piece.

Yet another aspect of the present invention is a culture system comprising: (a) a fluid inlet; (b) a first culture compartment having a tubular housing; (c) a first end piece attached to the fluid inlet on one side and to a first end of the tubular housing on a second side, (d) a second culture compartment coaxial with the first culture compartment, the second culture compartment having a proximal end and a distal end; (e) a fluid connector having a first side mounted on a second end of the tubular housing and a second side mounted on the proximal end of the second culture compartment, the fluid connector having a through bore passing from the first side to the second side of the fluid connector; (f) a connector filter positioned on the first side of the fluid connector to filter a fluid stream passing out of the first culture compartment and into the through bore of the fluid connector; (g) a fluid outlet; (h) a distal end piece mounted on the distal end of the second culture compartment and connected to the fluid outlet; and (i) an outlet filter transversing the second culture compartment including: a support cylinder having a first end supported by the fluid connector and a second end supported by the distal end piece, a

3

molecular weight cut-off membrane secured to an exterior surface of the support cylinder, and a chamber between the exterior surface of the cylinder and an interior surface of the membrane, the chamber in fluid communication with the through bore of the fluid connector and the fluid outlet.

Still yet another aspect of the present invention is a culture system comprising: (a) a fluid inlet; (b) a first culture compartment having a tubular housing; (c) a first end piece attached to the fluid inlet on one side and to a first end of the tubular housing on a second side, (d) a fluid connector having a first side mounted on a second end of the tubular housing, the fluid connector having a through bore passing from the first side to a second side of the fluid connector; (e) a connector filter positioned on the first side of the fluid connector to filter a fluid stream passing out of the first culture compartment and into the through bore of the fluid connector; (f) a culture bag including a flexible external wall having a first end, a second end, an internal side, and an external side, wherein the internal side of the external wall is positioned to face an interior of the culture bag, a first bag end fused to the first end of the external wall and attached to the second side of the fluid connector, a second bag end fused to the second end of the external wall, and an outlet filter supported by the second bag end; and (g) a fluid outlet.

Yet another aspect of the present invention is a culture system comprising: (a) a fluid inlet; (b) a first culture bag having a flexible external wall having a first end, a second end, an internal side, and an external side, wherein the internal side of the external wall is positioned to face an interior of the first culture bag, a first bag end fused to the first end of the external wall and attached to the fluid inlet, a second bag end fused to the second end of the external wall, and a first bag filter positioned on the second bag end to filter a fluid stream passing out of the first culture bag; (c) a fluid connector having a first side mounted on the second bag end, the fluid connector having a through bore passing from the first side to a second side of the fluid connector; (d) a second culture bag including a flexible outer wall having a first end, a second end, an internal side, and an external side, wherein the internal side of the outer wall is positioned to face an interior of the second culture bag, a proximal bag end fused to the first end of the outer wall and attached to the second side of the fluid connector, a distal bag end fused to the second end of the outer wall, and an outlet filter supported by the distal bag end; and (g) a fluid outlet.

The foregoing has outlined several aspects of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and the specific embodiments disclosed might be readily utilized as a basis for modifying or redesigning the method or process for carrying out the same purposes as the invention. It should be realized that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the present invention, and the advantages thereof, reference is now made to the following descriptions taken in conjunction with the accompanying drawings, in which:

FIG. 1 shows an oblique view of interlinked culture chambers;

4

FIG. 2 shows an oblique view of one embodiment of interconnected culture chambers of approximately equal capacity;

FIG. 3 is a longitudinal cross-sectional view of the interconnected culture chambers of FIG. 2;

FIG. 4 shows interconnected culture chambers where the chambers have different capacities and the larger chamber has a single diffusion membrane positioned within its interior;

FIG. 5 shows an oblique view of interconnected culture chambers where the chambers have different capacities and the larger chamber has multiple diffusion membranes positioned within its interior;

FIG. 6 is a longitudinal cross-sectional view of the interconnected culture chambers shown in FIG. 5;

FIG. 7 shows an oblique view of one embodiment of interconnected culture chambers of approximately equal capacity;

FIG. 8 is a longitudinal cross-sectional view of the interconnected culture chambers of FIG. 7;

FIG. 9 is one embodiment of interconnected culture chambers with an oblique view of the first chamber shown and a longitudinal cross-sectional view of the other chambers shown;

FIG. 10 is a longitudinal cross-sectional of a tandem bioreactor system wherein flexible disposable bioreactor chambers are mounted in tandem within a clamshell housing;

FIG. 11 is a longitudinal cross-sectional view of a dual conduit hydraulic flow swivel adapted for conveying two separate fluids into or out of a rotating bioreactor system;

FIG. 12 is a longitudinal cross-sectional of another tandem bioreactor system wherein flexible disposable bioreactor chambers are mounted in tandem within a clamshell housing; and

FIG. 13 is a longitudinal cross-sectional view of another tandem bioreactor system wherein flexible disposable bioreactor chambers are mounted in tandem within a clamshell housing.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The interlinked culture chambers of the present invention are designed for culturing cells, cellular aggregates, particles, tissues and organoids that respond to secretions from other cells, cellular aggregates, particles, tissues and organoids. The tandem culture chamber of the present invention has a first set of one or more biological culture chambers interlinked with a second set of one or more other biological culture chambers, wherein the secretions of the first set of biological culture chambers are fed into the second set of biological culture chambers without the intermingling of cells. Both reusable and disposable culture chambers are described for culturing cells, cell aggregates, particles, tissues and organoids.

The serially arranged culture chambers of the present invention have fluid-filled culture compartments in which cells, tissues and other biologicals are cultured. The walls of the chambers can be either rigid or a flexible bag-like construction. Normally, the bag construction is used in disposable applications in order to minimize possibilities of cross contamination between culture lots. Examples of suitable disposable bag-like chambers are described in U.S. Pat. No. 6,673,598 B1 issued on Jan. 6, 2004 and entitled "Disposable Culture Bag", which is hereby incorporated herein by reference. Examples of suitable rigid and bag-like

5

chambers are described in U.S. patent application, Ser. No. 10/725,607 filed Dec. 2, 2004 and entitled "Culture Chamber for Biologicals", which is hereby incorporated herein by reference.

In addition, either the rigid chamber or the bag system may use either a controlled molecular weight cut-off membrane structure or a controlled permeability filter to separate the culture media and the cells in the chamber from the outflow fluid. When a molecular weight cutoff membrane is used, certain constituents of the fluids on both sides of the membrane can diffuse across the membrane, so a properly constituted membrane can function to separate components of the biological media within the chamber from its outflow stream. When a suitably constructed outlet filter is positioned at the upstream end of the outlet end piece flow channel for a bioreactor chamber, the filter serves to prevent the cells and/or large particulate constituents of the biological media from passing through the filter into the outflow stream.

Different types of chambers may be used within an individual serially arranged culture system. For example, a rigid cell first chamber may be best suited for the preparation of a growth hormone, required nutrient, or precursor molecule needed for the well being of the cells in a disposable second chamber or vice versa. Interconnectors are described that allow multiple permutations of one or more rigid or flexible disposable culture chambers of a first set of chambers to be interlinked with one or more rigid or flexible disposable culture chambers of a second set of chambers.

The culture chambers of the present invention are generally configured so that they can be slowly rotated and provided with optimal conditions for the promotion of cell growth. The mechanisms for rotation of the bioreactors are not shown herein, but the details of the bioreactor chambers related to providing this rotational capability are shown. Accordingly, an example of an inlet and outlet fluid coupling swivel joint is described.

Interlinked Reusable Bioreactor Systems

The tandem culture chamber of the present invention has a first set of one or more biological culture chambers, hereinafter referred to as feeder cell chambers, interlinked with a second set of one or more other culture chambers. Reusable cell culture chambers are often desired to investigate a variety of systems for the production of a particular product and for optimizing certain growth parameters. Reusable cell culture chambers are typically made of a rigid plastic material that is non-toxic and non-reactive with biological systems, as well as being sterilizable.

The feeder cell chamber of the present invention has a fluid-filled culture compartment in which cells, tissues and other biologicals are cultured. The culture compartment is transversely by one or more molecular weight cut-off membranes attached to a membrane carrier assembly or provided with outlet filters to prevent the cells from leaving the culture compartment. Incoming nutrients and/or biological modifiers are transported through the membrane into the culture compartment and secretions (such as hormones, growth factors, differentiation factors, and metabolic waste products) are transported away from the fluid-filled culture compartment through the membrane and out the chamber outlet.

Referring now to the drawings, and initially to FIG. 1, it is pointed out that like reference characters designate like or similar parts throughout the drawings. The Figures, or drawings, are not intended to be to scale. For example,

6

purely for the sake of greater clarity in the drawings, wall thickness and spacing are not dimensioned, as they actually exist in the assembled embodiment. In the Figures used to describe the interlinked culture chambers the media flows from the left to the right. Thus, the first set of one or more chambers (i.e., the feeder cell chambers) is positioned on the left and the second set of chambers is positioned on the right.

EXAMPLE 1

Interlinked Reusable Culture Chambers

FIG. 1 illustrates the interlinking of a number of reusable culture chambers. Although numerous permutations of such arrangements are possible, FIG. 1 shows an arrangement connecting three separate rigid bioreactor vessels of the type described in detail in pending U.S. patent application, Ser. No. 10/725,607, entitled "Culture Chamber for Biologicals."

The first chamber **120** utilizes a first molecular cutoff membrane **137** and is upstream of the two bioreactors **130a,b** of the second chamber set. The first chamber **120** produces a desired growth hormone or other growth mediator for supply to the second chamber set **130a,b**. This arrangement is utilized when the cells grown in one bioreactor chamber **120** are able to provide sufficient growth hormone, precursor, or component to contribute to the growth of cells in multiple second bioreactor chambers **130a,b**. The second bioreactors **130a,b** may have the same cell line or different cell lines.

Each of the bioreactor chambers **120**, **130a,b** is rotated. A variety of drive assemblies may be used to rotate the culture chamber such as the drive assembly described in U.S. Pat. No. 6,080,581. The arrangement of the rotational equipment can be multiple independent sets of the standard rotators known to those in the industry, or alternatively a gang rotation system could be used. The bioreactor chambers **130a,b** are rotated substantially the same to generate a similar, balanced environment for the chambers. The two bioreactors of the second chamber set are similar to each other and use a second and third molecular cutoff membrane **189a,b** optimized for the cells being cultured in the bioreactors and for the products being produced by the second chamber set.

All three bioreactors shown in FIG. 1 are constructed of the same components except for their respective membranes and have most components in common with those used in the interconnected culture chambers shown in FIG. 2 and described in more detail in Example 2. Thus, each chamber has an end piece **11** sealingly engaged on each end of a sleeve **24** and also has a membrane carrier assembly **127**, located axially within the interior of sleeve **24**, thereby forming a bioreactor chamber. The sleeve **24** has multiple radial wall penetration ports **25**, as shown in FIG. 3. These penetration ports **25** are provided in the annular wall of sleeve **24** to allow the introduction of one or more fill ports **40** and one or more sampling ports **44**.

The biologicals being cultured in the rotatable chambers **120** and **130a,b** require nutrients, so fluid-conducting swivels **50**, stopcock valves **60**, intermediate tubings **173a,b** and fluid inlet tubing **172** and outlet tubing **171a,b** are provided for the system. The system **100** is plumbed so that nutrient fluid enters the first chamber **120** of the system through inlet tubing **172**, stopcock **60**, and swivel **50**. The fluid output from chamber **120** emerges through attachment neck **49** and swivel **50** of the outlet side endpiece **11** of the first chamber **120**.

The flow then passes to a fluid conducting cross fitting **151** which has connections to both the two bioreactor chambers **130a,b** via identical intermediate tubings **173a,b** and which also connects to another stopcock **60**. The intermediate tubings **173a,b** are identical in order to ensure the balanced flow of nutrients to the second set of chambers. The stopcock **60** on the cross fitting **151** is attached to secondary fluid feed line **174** which can be used to supplement or alter the fluid mixture delivered to the second chambers **130a,b**. The outflow from each of the second chambers passes through a swivel **50**, stopcock **60**, and outlet tubings **171a,b**. This arrangement permits full control of the fluid system for the bioreactors of system **100**.

EXAMPLE 2

Interconnected Equal Capacity Culture Chambers

FIG. 2 shows two interconnected bioreactors similar to the bioreactors described in Example 1. The particulars of the construction of the components of culture chamber **10** are best understood with reference to FIGS. 2–3. The serially arranged bioreactor system **10** is composed of two individual reusable rigid walled bioreactor chambers **20** and **30** cojoined by a fluid connector or center hub piece **80**.

The first bioreactor chamber **20** is located on the inlet side and serves to feed the second bioreactor chamber **30**. The media flows from the left to the right in the Figures. The biologicals being cultured in the rotatable chambers **20** and **30** require nutrients, so fluid-conducting swivels **50**, stopcock valves **60**, and fluid inlet tubing **72** and outlet tubing **71** are provided on the ends of the tandem housing **10** so that the entry and exit of media is controlled.

For first bioreactor chamber **20**, an end piece **11** seals to the upstream end of right circular cylindrical tubular sleeve **24** and center hub **80** seals to the downstream end so that a growth compartment **85** is formed within the enclosed space. When in use for culturing cells, cellular aggregates, particles, tissues and organoids, the culture chamber is designed to be supported on and rotated by a roller drive which rotates the chamber about its axis. In this situation, the end pieces **11** and the center hub **80** serve as tires for contact of the assembly **10** with the drive wheels of the drive assembly. A variety of drive assemblies, not shown here, may be used to rotate the culture chamber such as the drive assembly described in U.S. Pat. No. 6,080,581.

Multiple radial wall penetration ports **25** are provided in the annular wall of sleeve **24** to allow the introduction of one or more fill ports **40** and one or more sampling ports **44**. The swivel **50**, the fill port **40**, and the sampling port **44** are described in more detail in U.S. Pat. No. 6,673,598 B1 that is incorporated herein by reference.

End piece **11** has a right circular cylindrical central body having a coaxial push-on hose barb attachment neck **49** on its outer face. Axial through hole **15** penetrates through the attachment neck **49** and the rest of the body of end piece **11**. Reduced diameter coaxial right circular cylindrical interior projection **12** extends inwardly on the transverse face of the interior end of end piece **11**, with a flat-bottomed trepanned groove **14** located on the transverse interior face of end piece **11** immediately exterior of projection **12**.

The exterior cylindrical surface of interior projection **12** has annular male O-ring grooves. Elastomeric O-rings **18** are mounted in the O-ring grooves of projection **12**. At the interior end of interior projection **12** of end piece **11**, flat-bottomed coaxial counterbore **19** intersects through hole **15**. An optional lead-in chamfer may be provided at the

mouth of counterbore **19** in order to facilitate the stabbing of an O-ring seal with the membrane carrier assembly.

The right circular cylindrical sleeve **24** can be made of a variety of materials such as glass, stainless steel or plastic. Preferably the reusable cell culture chamber is constructed of plastic, typically a transparent plastic such as an acrylic plastic for the cylindrical sleeve **24** and opaque plastics such as Kynar™ or Delrin™ for the other rigid pieces such as the end pieces **11**. Suitable plastics have substantially zero porosity and are impermeable to gases and non-reactive to biological media and its components. Suitable construction materials must also be able to undergo multiple sterilizations by steam, gas, or radiation without deforming, cracking or otherwise being rendered unusable.

Although not shown in FIG. 3, the right circular cylindrical sleeve **24** is preferably provided with a lead-in taper on each of its interior corners to facilitate the stabbing of O-rings **18** over the interior projections **12** and **81** forming the ends of first chamber **20**. The interior bore of sleeve **24** is a close sliding fit to the outer diameter of interior projection **12** of end fitting **11**, thereby permitting O-rings **18** to sealingly engage the bore of sleeve **24**. O-rings **18** also serve to retain sleeve **24** in its desired axial position by virtue to their forceful frictional engagement with sleeve **24**.

As previously stated, the sleeve **24** has multiple radial wall penetration ports **25** to allow the mounting of fittings used for inserting fluid into or removing fluids from the growth compartment **85** and for allowing gas to escape from the growth compartment **85** as it is being filled with fluid. At each end of chamber **20**, sleeve **24** is stabbed over the interior projections **12** and **81** and bottomed out in the trepanned grooves **14** and **83** at the interior ends of end piece **11** and center hub **80**, respectively. The growth compartment **85** is located between the interior bore of the sleeve **24**, the membrane carrier assembly **27**, the interior end of interior projection **12** of end piece **11**, and the interior end of the interior projection **81** of the center hub piece **80**.

Center hub **80** is a structure composed of cylindrical segments and is symmetrical about its transverse midplane. Center hub **80** has an axial through hole **86** that provides communication from one side of the piece to the other, so that center hub **80** not only supports chambers **20** and **30**, but also provides a flow connection between the chambers **20** and **30**. The interior projection **81** of center hub **80** is identically structured to projection **12** of end piece **11** and carries two male O-ring grooves that hold O-rings **18**. The O-rings **18** mounted in the grooves of center hub **80** sealingly mate with and restrain the axial position of cylindrical sleeve **24**.

A membrane carrier assembly **27**, as shown in FIGS. 2 and 3, traverses the growth compartment **85**. An end of the generally cylindrical membrane carrier assembly **27** is mounted in the counterbores **19** and **82** of each of the two end assemblies **11** and **81**, respectively, used in chamber **20**.

FIG. 3 shows the details of construction of membrane carrier assembly **27**. Support cylinder **28** is symmetrical about its transverse midplane. The exterior of each end of cylinder **28** has, sequentially from its end, a lead-in taper to ease blind stabbing into a mounting hole, a first annular male O-ring groove **21** mounting elastomeric O-ring **87**, and a second groove **31** configured similarly to an O-ring groove and used to mount a second O-ring **87**, wherein the second O-rings serve to sealingly grip their respective ends of the membrane **37** which is deployed around the support cylinder **28**. Each end of cylinder **28** also has an axial blind hole **34** with multiple (in this case, four) equispaced coplanar radial cross holes **35** intersecting the inner end of blind hole **34**.

A small recessed surface pocket **32** having an arcuate cross-section is located on the exterior of cylinder **28** and is intercepted by each radial cross hole **35**. The depth of these surface pockets **32** below the outside cylindrical diameter is largest near the intersection with its cross hole **35** and linearly tapers to zero towards the middle of cylinder **28**.

Centrally deployed with a close fit around the exterior of cylinder **28** is a tubular molecular weight cut-off membrane **37**. Membrane **37** is flexible with a limited amount of elastic stretch capability. The construction of membrane **37** is very carefully controlled so that the number of molecules, having a molecular weight in excess of the specific limiting molecular weight cut-off value of the membrane **37**, diffused through the membrane in either direction is statistically very small and rapidly decreases as a function of increasing molecular weight. Thus, there is essentially no passage of molecules much larger than the molecular weight cut-off value of the membrane through the membrane **37**.

The molecular weight cut-off value of the membrane **37** is preselected so that nutrients and growth factors, as well as metabolic waste products, can easily diffuse through the membrane, whereas larger cellular products can be retained. For example, Factor VIII (having a molecular weight of about 350,000 daltons) or IgG monoclonal antibodies (having a molecular weight of about 155,000 daltons) produced by genetically engineered bacteria or cells can be retained by a membrane with a molecular weight cut-off value of about 100,000 daltons; whereas the majority of serum albumin (having a molecular weight of about 67,500 daltons and making up 55% to 62% of serum protein) would be allowed to pass through the membrane. Since the culture chamber **20** is reusable, the membrane carrier assembly **27** can be assembled with membranes **37** having a variety of molecular weight cut-off values depending on the desired secretions being produced. The membrane **37** is selected to allow the user to select a molecular weight cut-off value that would allow the desired protein or other cell secretions to serve as feedstock or stimulant for the cells in the second bioreactor chamber **30**.

As shown in FIG. 3, membrane **37** is sealed to the exterior of cylinder **28** at each end by using an O-ring **87** to circumferentially constrict over the exterior of membrane **37**, thereby forcing it into sealing engagement with groove **31** on the outside of cylinder **28**. In this manner, a small chamber in fluid connection with the radial flow passage **35** is formed between the exterior of surface pockets **32** and the interior of membrane **37**. The depth and length of cut for the surface pockets **32** is predetermined to be sufficient to produce a sufficient pressure area so that the elastic resistance of membrane **37** can be overcome. The expansion of the membrane **37** when media is passed through flow passage **35** and along the surface pockets **32** of support cylinder **28** permits a thin flow sheet of media between the membrane **37** and cylinder **28** to be established.

Second chamber **30** is a mirror image of first chamber **20** except that the membrane **89** is used in place of membrane **37** in the assembly of the membrane carrier assembly **27**. Membrane **89** is preselected for its molecular weight cut-off value according to desires of the user. The influx of fluid emerging from first chamber **20** and into the membrane carrier assembly **27** has its desired components diffusing across the membrane **89** into volume **88** for reaction with the cells therein. However, the bioreactive fluid within the volume **88** has waste products from the tandem cell arrangement of serial bioreactor **10** transfusing across membrane **89**

and into the outflow stream of chamber **30** to emerge through hole **15** in end piece **11** and thence to fluid outlet tubing **71**.

The present invention allows the user to select a membrane having a molecular weight cut-off value that would allow the desired protein or cellular product to pass out of the culture chamber with the waste products for collection and purification. However, the present invention also permits the user to select a membrane having a smaller molecular weight cut-off value than the desired protein or cellular product, so that the desired product is retained within the culture chamber and is concentrated with time as the cells multiply and continue to produce the desired product. Since the culture chamber **10** is reusable, the membrane carrier assemblies **27** can be assembled with membranes **37** and **89** having a variety of molecular weight cut-off values depending on the user's needs.

EXAMPLE 3

Interconnected Culture Chambers of Different Capacities

The interconnected culture chambers **10**, as shown in FIGS. 2 and 3, may be configured where either chamber is shorter than the other chamber thereby interconnecting culture chambers of different capacities but having a hub **80** that joins chambers of substantially the same internal diameter.

Alternatively, the interconnected culture chambers **300**, illustrated in FIG. 4, represent an embodiment of the serially arranged bioreactor system having culture chambers of different capacities. This bioreactor system utilizes basically the same arrangement as is used for the bioreactor system **10**, except that the second chamber is a different size either in diameter and/or in length than the first chamber.

This arrangement basically utilizes two rigid coaxial bioreactor vessels **320** and **330** cojoined in tandem. The first chamber **320** utilizes a first molecular cutoff membrane **37** and is upstream of the bioreactor **330** of the second chamber. One of the features of this embodiment is the large size of second chamber **330** relative to first chamber **320**. This arrangement is utilized when the first bioreactor chamber **320** is able to provide sufficient growth hormone or stimulator for a single large second bioreactor chamber **330**.

The cojoined bioreactor chambers **320** and **330** are rotated as an unit using standard rotational equipment known to those in the industry, but with three supporting drive rollers which act on the tires of the end pieces **11** and **311** and the center hub **380**.

The first chamber **320** produces a growth factor or other mediator for supply to the second chamber **330**. The bioreactor of the second chamber uses a second molecular cut-off membrane **337** that is optimized for its cells and the outputs desired from the second chamber.

The first bioreactor chamber **320** of the system embodiment **300** has all of its components including the input fluid system in common with that of the first chamber **20** of the interconnected culture chambers **10** of the present invention, except that a different fluid connector or center hub **380** is used. Thus, first chamber **320** has an end piece **11** sealingly engaged on the inlet end of a sleeve **24** and also has a membrane carrier assembly **27** located axially within the interior of sleeve **24**, thereby forming a bioreactor chamber as before. Multiple radial wall penetration ports **25** are

11

provided in the annular wall of sleeve 24 to allow the introduction of one or more fill ports 40 and one or more sampling ports 44.

The biologicals being cultured in the rotatable chambers 320 and 330 require nutrients, so fluid-conducting swivels 50, stopcock valves 60, fluid inlet tubing 72, and outlet tubing 71 are provided for the system. The system 300 is plumbed identically to that of the interconnected culture chambers of the bioreactor system 10 so that nutrient fluid enters the first chamber 320 of the system through inlet tubing 72, stopcock 60, and swivel 50. The fluid output from chamber 320 passes through a hole in center hub 380 into the second bioreactor chamber 330. The outflow from the second chamber 330 passes through a swivel 50, stopcock 60, and outlet tubing 71.

The growth compartment 385 of the first chamber 320 is located between the interior bore of the sleeve 24, the membrane carrier assembly 27, the interior end of interior projection of end piece 11, and the interior end of the interior projection 381 of the center hub piece 380.

Center hub 380 is a structure composed of right circular cylindrical segments. Center hub 380 has its first side on the first chamber side of its transverse midplane identical to the corresponding side of center hub 80 of the bioreactor system 10 and likewise identical to the end piece 11 of the first chamber 320 with hose barb attachment neck 49 removed. The first interior projection 381 of center hub 380 carries two male O-ring grooves that hold O-rings 18. The O-rings 18 mounted in the grooves of the interior projection of center hub 380 also sealingly mates with and restrains the axial position of cylindrical sleeve 24. Furthermore, the external cylindrical portion of hub 380 adjacent the interior projection serves as a tire for the rotation of the entire bioreactor assembly 300.

On its first side center hub 380 has a flat-bottomed cylindrical bore for housing the membrane carrier assembly 27 of the first chamber. On its second side obverse to the said first side of center hub 380 and in order from the transverse midplane of center hub 380 is a cylindrical flange 360 and a second projection 312 into the second chamber 330. The flange 360 is the same diameter as, or slightly larger than sleeve 324, used for the second chamber 330. The second interior projection 312 is sized to closely fit to the bore of sleeve 324 and has two male O-ring grooves mounting O-rings 318 to seal therewith.

An axial through hole provides communication from the counterbore on the first side of the hub 380 to a similar axial counterbore on the opposed side of hub 380. Optional lead-in chamfers are provided at the mouth of these counterbores in order to facilitate the stabbing of an O-ring seal with the membrane carrier assembly 327. Thus hub 380 not only supports chambers 320 and 330 but also provides a flow connection for the chambers.

Sleeve 324 is similar in construction to that of sleeve 24 used with the first chamber 320, but differs only in its inner and outer diameters. The inner diameter of sleeve 24 is chosen to be sufficient to accommodate the membrane carrier assembly 327 and provide sufficient volume for chamber 330. The outer diameter is selected for strength and rigidity. As is the case for sleeve 24, sleeve 324 has wall penetrations for the mounting of multiple fill ports 40 and sampling ports 44. Sleeve 324 is a close fit to second inner projection 312 of center hub 380.

End piece 311 has a right circular cylindrical central body having the same outer diameter as the enlarged flange having a coaxial push-on hose barb attachment neck 349 on its outer face. A reduced diameter coaxial right circular cylindrical

12

interior projection extends inwardly on the transverse face of the interior end of end piece 311, with a transverse shoulder located on the transverse interior face of end piece 311 immediately exterior of the projection. The exterior cylindrical surface of the interior projection 321 has annular male O-ring grooves. Elastomeric O-rings 318 are mounted in the O-ring grooves of projection 321. An axial through hole penetrates through attachment neck 349 and the rest of end piece 311, where it centrally intercepts a counterbored flat bottom hole to form a flow path for the fluid exiting from the second chamber 330. Optional lead-in chamfers are provided at the mouth of the counterbore in order to facilitate the stabbing of an O-ring seal with the membrane carrier assembly 327.

A membrane carrier assembly 27 as shown in FIG. 4 for this bioreactor system 300 traverses the growth compartment 385. An end of the generally cylindrical membrane carrier assembly 27 is mounted in the counterbores of each of the two assemblies 11 and 380, respectively, forming the end of compartment 385 used in chamber 320.

A membrane carrier assembly 327 as shown in FIG. 4 traverses the growth compartment 395 of the second chamber 330. The membrane carrier 327 is substantially identical to the unit used in the first chamber 385 and the prior embodiments 10 and 100 of the present invention, but membrane carrier 327 is much larger and mounts a different molecular cutoff membrane 337 that is optimized for the cell growth in the second chamber 330. An end of the generally cylindrical membrane carrier assembly 327 is mounted in the counterbores of each of the two chamber end pieces 311 and 380, respectively, forming the end of compartment 395 used in chamber 330.

EXAMPLE 4

Alternative Embodiment of Interconnected Culture Chambers of Different Capacities

The bioreactor system 200 has interconnected culture chambers arranged as shown in both FIGS. 5 and 6. This arrangement basically utilizes two rigid coaxial culture chambers 220 and 230 cojoined in tandem, and utilizes bioreactor vessel types substantially similar to those disclosed in U.S. patent application entitled "Culture Chamber for Biologicals", Ser. No. 10/725,607.

The first chamber 220 utilizes a first molecular cutoff membrane 37 and is upstream of the second chamber 230. One of the features of this embodiment is the very large size of second chamber 230 relative to first chamber 220. The second chamber 230 is designed to provide more molecular cutoff membrane active surface area than is present in the first chamber 220. This arrangement is utilized when the first bioreactor chamber 220 is able to provide sufficient secretions to stimulate and/or nurture the cells in a larger second chamber 230.

The cojoined bioreactor chambers 220 and 230 are rotated as an unit using standard rotational equipment known to those in the industry, but with three supporting drive rollers which act on the tires of the end pieces 11 and 211 and the center hub 280.

The first chamber 220 produces a desired growth factor or mediator for supply to the second chamber 230. The bioreactor of the second chamber 230 uses a second molecular cutoff membrane 237 that is optimized for the cells and the outputs desired from the second chamber 230.

The first bioreactor chamber 220 of the bioreactor system 200 has all of its components including the input fluid

13

system in common with that of the first chamber 20 of the bioreactor system 10 of the present invention, except that a different fluid connector or center hub 280 is used. Thus, first chamber 220 has an end piece 11 sealingly engaged on the inlet end of a sleeve 24 and also has a membrane carrier assembly 27 located axially within the interior of sleeve 24, thereby forming a bioreactor chamber as before. Multiple radial wall penetration ports 25 are provided in the annular wall of sleeve 24 to allow the introduction of one or more fill ports 40 and one or more sampling ports 44.

The biologicals being cultured in the rotatable chambers 220 and 230 require nutrients, so fluid-conducting swivels 50, stopcock valves 60, fluid inlet tubing 72, and outlet tubing 71 are provided for the system. The system 200 is plumbed similarly to that of serially arranged bioreactor system 300 so that nutrient fluid enters the first chamber 220 of the system through inlet tubing 72, stopcock 60, and swivel 50. The fluid output from chamber 220 passes through hole 286 in center hub 280 into the second bioreactor chamber 230. The outflow from the second chamber 230 passes through a swivel 50, stopcock 60, and outlet tubing 71.

The growth compartment 285 of the first chamber 220 is located between the interior bore of the sleeve 24, the membrane carrier assembly 27, the interior end of interior projection 12 of end piece 11, and the interior end of the interior projection 281 of the center hub piece 280.

Center hub 280 is a structure composed of right circular cylindrical segments. The first side of the center hub 280 facing the first chamber 220 is basically the same about its transverse midplane as the corresponding side of center hub 80 of the bioreactor system 10 and likewise identical to the end piece 11 of the first chamber 220 with hose barb attachment neck 49 removed.

The external cylindrical portion of hub 280 adjacent the interior projection 281 serves as a tire for the rotation of the entire bioreactor assembly 200. The first interior projection 281 of center hub 280 also carries two male O-ring grooves that hold O-rings 18. The O-rings 18 mounted in the grooves of projection 281 of center hub 280 sealingly mate with and restrain the axial position of cylindrical sleeve 24. On its first side center hub 280 has a flat-bottomed cylindrical bore 282 for housing the membrane carrier assembly 27 of the first chamber.

The second side of the center hub 280, obverse to the first side of center hub 280, has in order from the transverse midplane of center hub 280 a cylindrical flange 260 and second interior projection 261. Flange 260 is the same diameter as or slightly larger than sleeve 224 used for the second chamber 230. Second interior projection 261 is sized to closely fit to the bore of sleeve 224 and has two male O-ring grooves mounting O-rings 218 to seal therewith.

An axial hole 286 provides communication from the counterbore 282 on the first side of the hub 280 to approximately the middle of hub 280. Axial through hole 286 penetrates through interior projection 281 and approximately midway into the body of center hub 280 and is there branched into a number of radial holes 264. Offset from the axis of center of hub 280 are parallel holes 265, each of which centrally intercepts a counterbored flat bottom hole 263 and a corresponding radial hole 264 in order to form a flow path for the fluid entering the second chamber 230. The multiple counterbored holes 263 are positioned in a regular pattern on the transverse face 266 of the reduced diameter end 261 of center hub 280. Optional lead-in chamfers are provided at the mouth of counterbores 263 in order to facilitate the stabbing of an O-ring seal 229 with the mem-

14

brane carrier assembly 227. Thus hub 280 not only supports chambers 220 and 230 but also provides a flow connection between the chambers.

Sleeve 224 is similar in construction to that of sleeve 24 used with the first chamber 220, but differs only in its inner and outer diameters. The inner diameter of sleeve 224 is chosen to be sufficient to accommodate multiple membrane carrier assemblies 227 and provide sufficient volume for chamber 230. The outer diameter is selected for strength and rigidity. As is the case for sleeve 24, sleeve 224 has wall penetrations for the mounting of multiple fill ports 40 and sampling ports 44. Sleeve 224 is a close fit to inner projection 261 of center hub 280.

End piece 211 has a right circular cylindrical central body having the same outer diameter as the enlarged flange 260 having a coaxial push-on hose barb attachment neck 249 on its outer face. Reduced diameter coaxial right circular cylindrical interior projection 212 extends inwardly on the transverse face of the interior end of end piece 211, with a transverse shoulder 214 located on the transverse interior face of end piece 211 immediately exterior of projection 212. The exterior cylindrical surface of interior projection 212 has annular male O-ring grooves. Elastomeric O-rings 218 are mounted in the O-ring grooves of projection 212.

Axial hole 215 penetrates through attachment neck 249 and approximately midway into the body of end piece 211 where it is branched into a number of radial holes 216. Offset from the axis of end piece 211 are parallel holes 217, each of which centrally intercepts a counterbored flat bottom hole 219 and a corresponding radial hole 216 in order to form a flow path for the fluid exiting from the second chamber 230. Optional lead-in chamfers are provided at the mouth of counterbores 219 in order to facilitate the stabbing of an O-ring seal 229 with the membrane carrier assembly 227. The pattern of counterbores 219 is the mirror image of the counterbores 263 in the center hub 280.

A membrane carrier assembly 27 as shown in FIGS. 5 and 6 for this bioreactor system 200 traverses the growth compartment 285. The membrane carrier 27 is identical to the unit used in the bioreactor systems 10 and 100 of the present invention. An end of the generally cylindrical membrane carrier assembly 27 is mounted in the counterbores 19 and 282 of each of the two assemblies 11 and 281, respectively, forming the end of compartment 285 used in chamber 220.

A membrane carrier assembly 227 as shown in FIGS. 5 and 6 traverses the growth compartment 270 of the second chamber 230. The membrane carrier 227 is substantially identical to the unit used in the first chamber 285, but it mounts the same or a different molecular cutoff membrane 237 that is optimized for the cell growth and collection of the end product in the second chamber 230. An end of the generally cylindrical membrane carrier assembly 227 is mounted in the counterbores 219 and 263 of each of the two assemblies 211 and 280, respectively, forming the end of compartment 270 used in chamber 230.

EXAMPLE 5

Interlinked Reusable Culture Chamber with Cell Filter

Example 2 illustrated the interlinking of two reusable bioreactors, each of which was traversed by a membrane carrier assembly 27. This Example shows the interlinking of two reusable bioreactors, where one of the bioreactors is not traversed by a membrane carrier 27 but has a filter to prevent

15

the cells from flowing out of the bioreactor, while the media containing the cellular products does flow out of the bioreactor.

Referring to FIG. 7, the first bioreactor chamber 720 is located on the inlet side and serves to feed the second bioreactor chamber 730. Alternatively, the order of the bioreactor chambers 720 and 730 could be reversed so that the culture chamber 730 would be located on the inlet side to feed the second bioreactor chamber 720.

The bioreactor chamber 720 has an end piece 711 that seals to the upstream end of a right circular cylindrical tubular sleeve 724, while the center hub 780 seals to the downstream end of the sleeve 24 so that a growth compartment 795 is formed within the enclosed space. Multiple radial wall penetration ports 25 are provided in the annular wall of sleeve 724 to allow the introduction of one or more fill ports 40 and one or more sampling ports 44.

When in use for culturing cells, cellular aggregates, particles, tissues and organoids, the culture chamber is designed to be supported on and rotated by a roller drive that rotates the chamber about its axis as described for the interconnected culture chambers 10.

End piece 711 has a right circular cylindrical central body having a coaxial push-on hose barb attachment neck 749 on its outer face. Axial through hole 715 penetrates through the attachment neck 749 and the rest of the body of end piece 711. Reduced diameter coaxial right circular cylindrical interior projection 712 extends inwardly on the transverse face of the interior end of end piece 711, with a flat-bottomed trepanned groove 714 located on the transverse interior face of end piece 711 immediately exterior of projection 712.

The exterior cylindrical surface of interior projection 712 has an annular male O-ring groove. Elastomeric O-ring 718 is mounted in the O-ring groove of the projection 712. Although not shown in FIG. 8, the right circular cylindrical sleeve 724 is preferably provided with a lead-in taper on each of its interior corners. The tapers facilitate the stabbing of O-ring 718 over the interior projections 712 and the stabbing of O-rings 723 over the interior projection 781 forming the ends of first chamber 720.

At each end of chamber 720, sleeve 724 is stabbed over the interior projections 712 and 781 and bottomed out in the trepanned grooves 714 and 782 at the interior ends of end piece 711 and the fluid connector or center hub 780, respectively. The interior bore of sleeve 724 is a close sliding fit to the outer diameter of interior projection 712 of end fitting 711, thereby permitting O-ring 718 to sealingly engage the bore of sleeve 724. O-ring 718 also serves to retain sleeve 724 in its desired axial position by virtue to its forceful frictional engagement with sleeve 724.

Center hub 780 is a structure composed of cylindrical components. The first side of center hub 780 has the interior projection 781 projecting toward the chamber 720. The second side of center hub 780 has the interior projection 721 projecting toward the chamber 730. An axial through hole 786 provides communication from one side of the hub to the other side, so that center hub 780 not only supports chambers 720 and 730, but also provides a flow connection between the chambers 720 and 730.

Reduced diameter coaxial right circular cylindrical interior projection 781 has a flat-bottomed trepanned groove 782 located on the transverse interior face of the center hub 780 immediately exterior of projection 781 into which the sleeve 724 fits. The exterior surface of the projection 781 carries two male O-ring grooves that hold O-rings 723. The O-rings

16

723 mounted in the O-ring grooves of center hub 780 sealingly mate with and restrain the axial position of cylindrical sleeve 724.

The projection 781 has a coaxial flat-bottomed counterbore 755 in its interior transverse end. A controlled porosity cell filter 750, which has a thickness less than the depth of the counterbore 755, is inserted into a flat-bottomed counterbore 755 to be externally flush with the top of the counterbore 755 thereby creating a collector plenum 757 between the filter 750 and the counterbore 755. The filter 750 allows the flow of media and nutrients from one chamber to the other chamber, but it prohibits cells or particles from leaving the chamber in which they are being cultured.

Reduced diameter coaxial right circular cylindrical projection 721 is substantially similar to the interior projection 82 of hub 80. The projection 721 has a flat-bottomed trepanned groove 783 located on the transverse interior face of the center hub 780 immediately exterior of projection 721 into which the sleeve 24 fits. The exterior surface of the projection 721 carries two male O-ring grooves that hold O-rings 18. The O-rings 18 mounted in the O-ring grooves of center hub 780 sealingly mate with and restrain the axial position of cylindrical sleeve 24.

A membrane carrier assembly 27, described above, traverses the bioreactor chamber 730 and its growth compartment 785. The interior end of the membrane carrier assembly 27 is mounted in the counterbore 762 of the projection 721. The other end of the membrane carrier assembly 27 is mounted in the counterbore of the end piece 11 that forms the outside end of the bioreactor chamber 730.

EXAMPLE 6

Interlinked Reusable and Disposable Culture Chambers

Example 1 illustrated the interlinking of multiple reusable culture chambers. This Example shows one alternative configuration of interlinked reusable and disposable culture chambers. The bioreactor system 800 has serially arranged biological culture chambers as shown in FIG. 9. The arrangement of the system uses a single, separate rigid first chamber 820 with its outflow stream split and delivered to two independent, separate disposable bag second chambers 845. The second chambers 845 have provision for secondary nutrient streams to be added to the outflow stream from the first chamber.

Referring to FIG. 9, first chamber 820 has an essentially identical construction with that of chamber 120 of the bioreactor system 100 of the present invention. The first chamber 820 utilizes a first molecular cut-off membrane 137 and is upstream of the two culture chambers 845 of the second chamber set. The individual second chambers 845 are soft-sided disposable culture bags described in U.S. Pat. No. 6,673,598 and incorporated herein by reference.

FIG. 9 does not show the split cylindrical bag housing needed to support the culture bags 845 in order to see the culture chambers 845 more clearly. However, similar versions of this hardware are described below and in U.S. Pat. No. 6,673,598. Each of the bioreactor chambers 120 and 845 is rotated. The arrangement of the rotational equipment can be multiple independent sets of the standard rotators known to those in the industry, or alternatively a gang rotation system could be used. However, typically the bioreactor chambers 845 will be rotated identically so that a similar,

balanced performance can be expected from the biological reactions within each chamber.

The first chamber **820** produces a desired growth factor or mediator for supply to the second set of chambers **845**. The two bioreactors of the second chamber set may vary in configuration depending upon the desired used. The second chamber set, illustrated in FIG. 9, are identical to each other and use a molecular cutoff filter **886** optimized for the cells and the collection of end product from the second chamber set.

The bioreactor of the first chamber **820** has an end piece **11** sealingly engaged on each end of a sleeve **24** and also has a membrane carrier assembly **127** located axially within the interior of sleeve **24**, thereby forming a bioreactor chamber as before. Multiple radial wall penetration ports **25** are provided in the annular wall of sleeve **24** to allow the introduction of one or more fill ports **40** and one or more sampling ports **44**.

The biologicals being cultured in the rotatable chambers **820** and **845** require nutrients, so fluid-conducting swivels **50** and **869**, stopcock valves **60**, intermediate tubing **173a,b**, and fluid inlet tubing **172** are provided for the system. The system **800** is plumbed so that nutrient fluid enters the first chamber **820** of the system through inlet tubing **172**, stopcock **60**, and swivel **50**. The fluid output from chamber **820** emerges through attachment neck **49** and swivel **50** of the outlet side endpiece **11** of the first chamber **820**. The flow then passes to a fluid conducting cross fitting **889** which has connections to both the two second culture chambers **845** via identical intermediate tubing **173a,b** and which also connects to another stopcock **60**. The intermediate tubings **173a,b** are generally identical in order to ensure the balanced flow of nutrients to the second chambers **845**. The stopcock **60** on the cross fitting **889** can be used to control the flow of the fluid mixture delivered to the second chambers **845**. The outflow from each of the second chambers passes through a swivel, a stopcock (not shown), and outlet tubings (not shown). This arrangement permits full control of the fluid system for the bioreactor system **800**.

The second bioreactor chambers **845** utilize disposable flexible bags, in this instance bag assemblies **846**. During fabrication of the bag assemblies **846**, the end of a bag **847** will be sealingly welded or fused to the external cylindrical surfaces of the inlet **850** and outlet **880** bag ends.

To assist in mixing the fluid in the bag assembly **846**, one or more optional perfusion tubes will be incorporated into the bag assembly. Two types of perfusion tubes, wall tubes **848** and internal tubes **849**, are used for the purpose of introducing nutrients into the interior volumes of the second set of culture chambers **845**. In the cross-sectional view of FIG. 9, the wall perfusion tubes **848** are mounted on the interior of each bag wall. Each wall perfusion tube **848** is supported over its length, beginning a small distance from the inlet plenum **851** of inlet bag end **850**, by being joined integrally with a lapped bag seam. The perfusion tube **848** is symmetrically positioned in the seam and fused to the wall of bag **847** by welds on each side of seam. The weld joints only cover a portion of the circumference of the wall perfusion tubes **848**, so that a substantial portion of each tube is still exposed to the interior volume of the bag **847**.

The bag assembly **846** may also have one or more end supported internal perfusion tubes **849** of various configurations. Example configurations are shown in FIGS. 9, 10 and 12. The internal tubes **849**, shown in FIG. 9, extend from the inlet transverse end cap **858** into the interior of the bag assembly **846**. The internal perfusion tubes **848** and **849** will vary in length, weight and flexibility, depending on the

viscosity of the media. The internal perfusion tubes **848** and **849** move with the rotation of the bag support assembly **846**, thus adding to the mixing of the media within the bag. Additionally, end supported tubes **849** (two shown) extend from the inlet plenum **851** to the other side of the bag **847**, where they are anchored at their tips by welding to the inside of the bag. The end supported internal tubes **849** are branched to further aid perfusion and mixing within the bag assembly **846**.

Each perfusion tube **848** and **849** has a distal closure **866** where the perfusion tube is closed or sealed by crimping and/or fusing. In contrast, the inlet end of each perfusion tube **848** and **849** is in communication with the fluid inlet plenum **851** for the bag assembly **846**. The material of construction of perfusion tubes **848** and **849** is a biologically-compatible, non-toxic, non-protein-absorbing, flexible polymer having a number of perforations or a controlled permeability for the nutrient fluids for the biological media of the second chamber assembly **845**, allowing fresh media to be pumped into each bag assembly **846** under a low pressure gradient, similar to a soaker hose used in watering lawns.

The exterior of the body of each inlet bag end **850** is a relatively thin walled right circular cylinder that is sized to have a close fit within the bag holder of its rotating assembly (not shown). On the interior end of inlet bag end **850** is located thin transverse inlet end cap **858**. On the exterior end of inlet bag end **850** and adjoined to it by a transverse shoulder is reduced diameter cylindrical extension connecting neck **859**, which has a coaxial through hole **853** penetrating into plenum **851**. The exterior of connecting neck **859** has, in order from its attachment to bag inlet end **850**, a first cylindrical section, an annular triangular cross-section latching detent with a transverse shoulder on its inner side, and a second cylindrical section having the same diameter as the first and mateable with a female O-ring. Inlet end cap **858**, the outer transverse end of cylindrical inlet bag end **850**, and the interior of the cylinder of inlet bag end **850** form inlet plenum **851**.

The outflow stream from first chamber **820** freely passes by way of an outlet swivel **50** to cross fitting **889**, branching there to intermediate tubings **173a,b** and thence via dual input swivels **869** to the inlet plenums **851** for distribution into the interior of bag assemblies **846** through the perfusion tubes **848** and **849**.

Multiple radial branch ports extend through the cylindrical wall of inlet bag end **850** and intersect inlet plenum **851**. An attached outer perfusion tube **848** is fused into each branch port so that the bore of each tube **848** is in communication with inlet plenum **851**.

Likewise, multiple interior perfusion tubes **849** are fused into corresponding branch ports that are provided in inlet end cap **858** coplanar with and at an angle ranging from 0° to 45° to the axis of the inlet end cap. Each end supported interior perfusion tube **849** thus has its bore in communication with inlet plenum **851**.

Outlet bag end **880** is a right circular cylinder that has a coaxial flat bottom counterbore **881** in its interior transverse end and a connecting neck **882** for insertion into a receiving socket of a swivel **50**. Concentric through bore **883** passes through neck **882** and intersects counterbore **881**.

A controlled porosity cylindrical filter disk **886**, which has a thickness less than the depth of counterbore **881**, is sealingly mounted externally flush in counterbore **881**, thereby creating collector plenum **887** between the filter disk **886** and the counterbore **881**. Outflow fluid emerging from the second chamber **845** is filtered by disk **886** to prevent the

19

cells within the second chamber from leaving that chamber, while at the same time waste products and the desired output products produced within second chamber **845** are passed by filter disk **886**. A fluid swivel **50** is then attached to the connecting neck **882** of bag end **880** to convey the outflow from the second chamber system **845** to storage or processing.

The dual input swivel **869** serves both to commingle the outflow from the first bioreactor **820** with a separately supplied secondary nutrient stream and to convey that fluid through a rotating connection with the connection neck **859**. The body of swivel **869** is cylindrical with transverse ends and a coaxial bore **860** that provides a close fit to the cylindrical portion of connection neck **859**. From its interior end, the bore **860** has a female O-ring groove housing an O-ring that sealingly mates with the rotatable connection neck **859** of the bag assembly **846** and then a triangular cross-section annular groove with a transverse outer end. When the input swivel **869** is stabbed onto the connection neck **859**, the annular ridge of the connection neck engages into the groove in the bore **860** so that the two parts are retained together. Dual input swivel **869** has two identical hose connection barbs **854** which are diametrically opposed on the outer cylindrical surface of the swivel body.

Coaxial through holes **855** pass through the barbs **854** and penetrate into the bore **860** of the swivel **869**. For each of the two swivels **869**, one barb **854** is attached to an intermediate tubing **173a** or **173b**, while the other is optionally attached to the tubing (not shown) that supplies the secondary nutrient stream to the swivel. If the other barb **854** is not attached to a secondary nutrient stream it is capped.

Interconnected Disposable Bioreactor Bags

Disposable culture bags, or bioreactor bags, are needed by the biotechnology industry to cut down on the time and expense of cleaning, sterilizing and verifying bioreactors with each new batch of product.

The present invention includes a reusable bag housing to support interconnected disposable culture chambers such as the bioreactor system **900** shown in FIG. **10**. Since the bag assemblies are designed to be supported by a bag support housing, very large capacity bag assemblies can be used to scale-up production procedures. The bag assemblies described herein are made to hold anywhere from milliliters to thousands of liters of fluid. Furthermore, the bag assemblies described herein are pre-sterilized before use, typically done using gamma radiation or gas.

The bioreactor bags are housed in a rotatable housing and rotated by a drive mechanism as described in pending U.S. Pat. No. 6,673,598 B1. The disposable bioreactor bags have rigid end pieces that can be configured to carry one or more membrane carrier assemblies **27** with molecular weight cut-off membranes **37**.

EXAMPLE 7

Interconnected Disposable Culture Chambers

The bioreactor system **900** has serially arranged biological culture chambers as shown in FIG. **10**. This arrangement of the system uses a disposable flexible bag assembly **901** for the first chamber. However, multiple disposable bag assemblies **946** mounted in parallel are used for the second chamber system. The bags used for the second chamber system are constructed with multiple diffusion tubes and an outlet filter. All of the bags are mounted in a single cylin-

20

drical bag support housing **930** for rotation as a unit. This particular arrangement is desirable when the interior volumes of the second chamber bags **946** must be kept relatively small.

The arrangement shown in FIG. **10** is also desirable when different types of end products are desired from different cells being simultaneously grown in the system. In this latter case, the outputs of the second chamber system would not be commingled as shown in FIG. **10**, but would flow through a dual conduit hydraulic swivel **400**, shown in FIG. **11**, coaxially mounted on bag support housing **930** so that the outputs of the second chamber bags would be maintained separately.

Alternatively arrangement of the bioreactors shown in FIG. **10** may be switched so that there are multiple chambers **946** on the first side and the chamber **901** on the second side. This arrangement would be advantageous if a cell line required growth factors or products produced by more than one cell type.

The type of chambers (with or without membrane carrier assemblies, perfusion tubes, or both) used in the bioreactor system can be varied as desired. Furthermore, varying the width or the length of the chambers can vary the size of the chambers. The housing **930** is configured to support and rotate the bioreactor system designed by the investigator to meet specific growth requirements.

Referring to FIG. **10**, bag assembly **901** is constructed to support, by means of mounting in bag ends **905** and **921**, multiple membrane carrier assemblies **27** within the interior of the bag **920**. The bag assembly **901** includes bag **920**, two end pieces **905** and **921** for establishing fluid interconnections and positioning the bag assembly **901**, and the multiple membrane carrier assemblies **27**.

Bag assembly **901** includes multiple service ports such as a gas venting port **44** and a fill port **40**. The fill port **40** is bonded into a penetration in the side of bag **920**, and the sampling port **44** also is bonded into a penetration in the side of bag **920**. The attachments of ports **44** and **40** into bag **920** are reinforced by sealing grommets on both sides of the bag wall, as is well understood by those skilled in the art. Additionally, a quick fill port **970** is inserted into a reinforced hole in the wall of bag assembly **901** and welded in so that it has a position parallel to and offset from the axis of bag assembly **901**. The bag assembly **901** has its constituent components welded or bonded or fused together. Furthermore, all of the components of the bag assembly **901** contacting the biological media are biologically non-reactive, non-toxic and exhibit low protein binding properties.

Bag inlet end **905** is a right circular cylindrical disk having a concentric connecting neck **904** on its outer transverse face. Connecting neck **904** has, in sequential order from the outer face of bag end **905**, a straight cylindrical shank, an externally projecting frusto-conical latching shoulder, and a second straight cylindrical outer shank, so that it can be latched into swivel **50**. Coaxial hole **910** is located on the axis of connecting neck **904** and penetrates through the length of connecting neck **904** and part way into the main cylindrical body of bag end **905**. Hole **910** is intersected by multiple equispaced radial cross holes **912** which extend from the central axis of bag inlet end **905** approximately 90 percent of the distance to the outer cylindrical face.

The interior transverse end of bag interior end **905** has multiple angularly equispaced flat-bottomed counterbores **914** located in multiple concentric ring patterns. Each counterbore **914** is intercepted by the short coaxial blind holes **913**, which are in turn intersected by the radial cross holes

21

912. Accordingly, a fluid path is created from the hole 910 transversing connecting neck 904, through the cross holes 912 and the short connecting holes 913 into each of the counterbores 914. The diameter of counterbore 914 is such that it provides a close fit to the outer diameter of the inlet end of the membrane carrier assembly 27.

Bag outlet end 921 is structured very similarly to bag inlet end 905 in general and is basically identical to its interior end. The bag outlet end 921 is a right circular cylindrical disk having multiple flat bottom counterbored sockets 922 on its outer transverse face in a single concentric regular circular array. Each of the sockets 922 has a short coaxial connecting hole 923 which is intercepted by a radial hole segment 924 which extends from its hole 923 to the center axis of bag outlet end 921, where it intersects short axially positioned central hole 925. Coaxial hole 925 is intersected by multiple equispaced radial cross holes 926 which extend from the central axis of bag outlet end 921 approximately 90 percent of the distance to the outer cylindrical face.

The interior transverse end of bag outlet end 921 has multiple angularly equispaced flat-bottomed counterbores 927 located in multiple concentric ring patterns identical to those in bag inlet end 905. Likewise, the pattern of radial holes 926 is identical to that of radial holes 912 of bag inlet end 905. Each counterbore 927 is intercepted by the short coaxial blind holes 928, which are in turn intersected by the radial cross holes 926. Accordingly, a fluid path is created from the counterbores 927, holes 928, through the radial cross holes 926 and the short connecting holes 925 and 923 into each of the counterbored sockets 922.

The diameter of the counterbores 922 is such that they provide a close fit to the outer diameter of the exit end of the support cylinder of the membrane carrier assembly 27. At bag assembly, the bag inlet end 905 and the bag outlet end 921 have their respective counterbores 914 and 927 aligned and the membrane carrier assemblies 27 are all sealingly inserted and bottomed in their socketing counterbores 914 and 927. The bag ends 905 and 921 are then circumferentially welded or otherwise fused on their outer cylindrical faces to the corresponding ends of bag 920 at the end openings. The length of the bag assembly is chosen to accommodate the membrane carrier assemblies used.

The membrane carrier assembly 27 transversing the bag assembly 900 has been previously described and is shown in detail in FIGS. 2 and 3. The first O-rings 87 provide a seal between the counterbores 914 and 927 and the carrier assembly 27. The membrane 37 is retained in place over the outer diameter of the central portion of support cylinder 28 by the use of second O-rings 87 to seal and anchor each end of membrane 37 into each groove 31.

The second bioreactor chamber utilizes multiple disposable flexible bags, in this instance multiple bag assemblies 946, mounted within the support housing 930 between its outlet end bulkhead 938b and its internal diaphragm 936. Each of the bag ends 950 and 980 are respectively supported by the counterbored sockets 922 on the outlet transverse side of outlet bag end 921 and in the counterbored sockets 985 of the bag header 988, respectively. During fabrication of the second bag assemblies 946, the ends of bags 947 will be sealingly fused or welded to the external cylindrical surfaces of the inlet 950 and outlet 980 bag ends.

To assist in mixing the fluid in the bag assembly 946, one or more optional perfusion tubes will be incorporated into the bag assembly. Three types of perfusion tubes 948, 949, and 957 are used for the purpose of inducing nutrients into the interior volumes of second bioreactor chamber system 945. In the cross-sectional view of FIG. 10, multiple outer

22

perfusion tubes 948 are mounted on the interior of each bag wall. Each outer perfusion tube 948 is supported over its length, beginning a small distance from the inlet plenum 951 of inlet bag end 950, by being joined integrally with a lapped bag seam. The perfusion tube 948 is symmetrically positioned in the seam and fused to the wall of bag 947 by welds on each side of seam. The weld joints only cover a portion of the circumference of the outer perfusion tubes 948, so that a substantial portion of the tubes is still exposed to the interior volume of the bag 947.

The bag assembly 946 may also have one or more cantilevered internal perfusion tubes 949 of various configurations. As seen in FIG. 10, tubes 949 extend from the inlet transverse end cap 958 into the interior of the bag 947. The internal perfusion tubes 948 and 957 will vary in length, weight and flexibility, depending on the viscosity of the media. The internal perfusion tubes 948 and 957 move with the rotation of the bag support assembly 946, thus adding to the mixing of the media within the bag. Additionally, end supported tubes 957 (two shown) extend from the inlet plenum to the other side of the bag 947, where they are anchored at their tips by welding to the inside of the bag.

Each perfusion tube 948, 949, and 957 has a distal closure 960 where the perfusion tube is closed or sealed by crimping and/or fusing. In contrast, the inlet end of each perfusion tube 948, 949, and 957 is in communication with the fluid inlet plenum 951 for the bag assembly 946. The material of construction of perfusion tubes 948, 949, and 957 is a biologically-compatible, non-toxic, non-protein-absorbing, flexible polymer having a number of perforations or a controlled permeability for the nutrient fluids for the biological media of the second chamber assembly 945, allowing fresh media to be pumped into each bag assembly 946 under a low pressure gradient, similar to a soaker hose used in watering lawns.

The exterior of the body of each inlet bag end 950 is a relatively thin walled right circular cylinder that is sized to have a close slip fit within its mating flat bottom counterbored socket 922 of the outlet bag end 921 of the first bag assembly 901. On the interior end of inlet bag end 950 is located thin transverse inlet end cap 958. On the exterior end of inlet bag end 950 is reduced diameter cylindrical extension 959, which has a coaxial through hole 923 penetrating into plenum 951. The exterior of cylindrical extension 959 has a male O-ring groove with an O-ring to seal to the bore of socket 922 of the outlet bag end 921 of the first chamber 901. Inlet end cap 958, the inner end of cylindrical extension 959, and the interior of the cylinder of inlet bag end 950 form inlet plenum 951. The flow stream from the first chamber 901 freely passes to the inlet plenum 951 for distribution into the interior 945 of bag assembly 946 through the tubes 948, 949, and 957.

Multiple radial branch ports extend through the cylindrical wall of inlet bag end 950 and intersect inlet plenum 951. An attached outer perfusion tube 948 is fused into each branch port so that the bore of each tube 948 is in communication with inlet plenum 951. Likewise, multiple interior perfusion tubes 949 and 957 are fused into corresponding branch ports that are provided in inlet end cap 958 coplanar with and at an angle ranging from 0° to 45° to the axis of the inlet end cap 958. Each cantilevered interior perfusion tube 949 and 957 thus has its bore in communication with inlet plenum A 951.

Outlet bag end 980 is a right circular cylinder that has a coaxial flat bottom counterbore 981 in its interior transverse end and a connecting neck 982 for insertion into a receiving

23

socket **989** of bag header **988**. Concentric through bore **983** passes through neck **982** and intersects counterbore **981**.

Controlled porosity cylindrical filter disk **986**, which has a thickness less than the depth of counterbore **981**, is sealingly mounted externally flush in counterbore **981**, thereby creating collector plenum **987** between the filter disk **986** and the counterbore **981**. Outflow fluid emerging from the interior of the second chamber **945** is filtered by disk **986** to prevent the cells within the second chamber from leaving that chamber, while at the same time waste products and the desired output products produced within the second chamber are passed by filter disk **986**.

Bag header **988** is a thick circular disk that has an equispaced circular pattern of flat bottom counterbored sockets **985** located in its interior transverse face. The pattern for sockets **985** is the same as the pattern of sockets **922** in the outer face of outflow bag end **921** of the first bag assembly **901**. A short hole **990** coaxial with each socket **985** penetrates approximately 60 percent of the way through bag header **988**, where it intersects one of an array of radial holes **991** which extend from short hole **990** to the center axis of the bag header. Hole **992** extends outwardly along the axis of bag header **988** from the intersections of the radial holes **991** through outwardly extending coaxial connecting neck **995**. Functionally, connecting neck **995** is substantially similar to connecting neck **904** of the first bag assembly **901**, so it has an annular latching ridge to effect a latching with an outlet fluid swivel **50**. A fluid swivel **50** (not shown) is then attached to the connecting neck **995** of bag header **988** to convey the outflow from the interior of the second chamber system **945** to storage or processing.

In addition the bag assemblies **901** and **946** are provided with a unique identifier **995**. This identifier **995** may be a bar code, a magnetic strip or any other identifier to enable the user of the bioreactor system to easily track the bioreactor bags and the cells, cellular aggregates, particles, tissues or organoids and their end products through growth and processing.

Although the bag support housing **930** may be made any shape, the bag support housing is preferably a circular cylinder to ease its horizontal rotation. Bag support housing **930** is a circular cylinder having transverse end bulkheads **938a,b**. The arrangement used for this embodiment **900** is similar to the unit **530** shown in FIG. 12 with the bioreactor system **500**. Bag support housing assembly **930** (having certain features shown in more detail in FIGS. 3-5 of U.S. Pat. No. 6,673,598) is hollow so that its cylindrical wall has a uniform wall thickness.

Bag support housing **930** is provided with an internal diaphragm **936** that serves to support the outlet end of the first bioreactor chamber **901**. Preferably bag support housing **930** has a diametrical split so that it is divided into a top portion and a bottom portion, where the top and bottom portions are hinged together on one cylindrical side of the diametrical split and a latch is provided on the opposed side of the diametrical split. The hinge allows the top portion and the bottom portion of the bag support assembly **930** to be selectively opened and closed.

Central concentric bores are located in both the transverse inlet and outlet bulkhead ends **938a,b** and the central diaphragm **936** of the bag support assembly **930** and are designed to fit around the bag ends **905** and **921** and the bag header **988** of the second bioreactor bag assembly **946**, while additional offset bores are located on the diametrical split so that they can readily accommodate insertion of fill ports into the split bore. One or more additional radial ports with clearance to permit insertion of a corresponding number of

24

gas removal ports are appropriately positioned in the cylindrical walls of the upper and lower portions of the bag support housing **930**. The drive assembly (not shown) rotates the bag support assembly **930** so that the axis of the bag support assembly and its contained bags is horizontal.

One advantage of bag support housing **930** is that the bag assemblies **901** and **946**, which are made with their inlet and outlet connections incorporated into the bag assembly so that they can be coupled and also preplumbed to their respective inlet **72** and outlet **71** tubings, can be inserted into the bag support housing **930** without having to disconnect any tubing or connectors. A drive assembly (not shown) horizontally rotates the bag support assembly **930**.

EXAMPLE 8

Interconnected Disposable Culture Chambers with Externally Added Media

Alternative bioreactor systems not only allow secretions from a first culture chamber to flow into a second culture chamber, but they also allow externally added media and nutrients to be added to the second culture chamber. In order to allow media to be added to the second culture chamber in controlled proportions with the culture secretions from the first chamber, a dual conduit hydraulic swivel **400** is used as shown in FIG. 11.

Swivel **400** consists of a cylindrical outer body **401** and a core piece **440**. Outer body **401** has an axially projecting hose barb **402** on its first end, with axial first flow hole **403** extending through the barb and into first interior cylindrical cavity **407**. Triangular cross-section external annular ridge **406** is located on barb **402** to sealingly and axially engage an attached hose.

First interior cylindrical cavity **407** has a centrally positioned female O-ring groove **408** mounting O-ring **409**. Cavity **407**, at the end opposed to the hose barb **402** side, has a conical transition where it intercepts a coaxial second interior cylindrical cavity **412**. The second interior cylindrical cavity **412** has a female O-ring groove **413** mounting O-ring **414** and an annular latch groove **415** having right triangular cross-section wherein the transverse shoulder of groove **415** is on the exterior end of cavity **412**. Furthermore, a radial hose barb **418** extends off of the outer body **401**. A second axial through flow hole **419** penetrates through the hose barb **418** and into the second cavity **412** inside of the O-ring groove **413**. The radial hose barb **418** has a triangular cross-section external annular ridge **420** to engage an attached hose.

The body of core piece **440** is basically a stepped cylinder with a central axial through hole **441** that serves as a coaxial extension of first flow hole **403**. Core piece **440** is fully inserted into the bore of outer body **401**. From its inner end which is inserted into the interior of outer body **401**, the exterior of core piece **440** has a taper which permits stabbing into O-ring **409**, a first cylindrical section **442** closely fitting to cavity **407**, a conical transition shoulder which permits stabbing into O-ring **414**, and a second cylindrical section **443** closely fitting to cavity **412**. Core piece **440** has a coaxial annular triangular cross-section latching ridge **444** outward of O-ring groove **413** in a position where it can readily mate with and latch with latch groove **415** and thus hold the swivel **400** in proper axial alignment.

Radial port **448** intercepts shallow annular external groove **447** on cylindrical section **443** and both are located in the same transverse plane as second radial hose bard **418**. Third flow hole **449** is parallel to and offset from axial hole

25

441 of core piece 440. Third flow hole 449 extends from the exterior end of core piece to intercept radial port 448. Third flow hole is plugged with insert plug 460 at its outer end. Radial hose barb 451 has an axial fourth flow hole 450 intercepting into third flow hole 449. O-rings 409 and 414 and plug insert 460 isolate a continuous flow path consisting of the radial port 440, the second 419, third 449, and fourth 450 flow holes and groove 447. Hose barb 451 has a triangular cross-section external annular ridge 452 to engage an attached hose.

The external end of core piece 440 of swivel 400 is mounted into mounting flat bottom counterbore 471 centrally positioned in a bioreactor bag end 470, where it is glued or welded at circumferential joint 445. Bioreactor bag end 470 has a short axial hole 472 communicating with counterbore 471 on its outer end and radial distributor holes 473 on its inner end.

A primary or first fluid path consisting of first flow hole 403 of outer body 401, axial hole 441 of core piece 440, and hole 472 of cell end piece 470 thus is created from hose barb 402 into the distributor holes 473 of cell end 470. An independent secondary fluid flow path consisting of the second flow hole 419 of outer body 401, the radial port 448, the third flow hole 449 and fourth flow hole 450 with groove 447 of core piece 440 is also provided. Thus the swivel 440 can handle a flow stream entering through the primary flow path, as well as the secondary flow path. This dual conductor hydraulic swivel arrangement 400 can thus be used not only to provide a primary nutrient stream to the upstream first chamber of a coaxially positioned and serially tandem mounted reactor system, but the secondary flow stream can be used to augment or otherwise modify the flow stream from the first chamber to the second chamber of the bioreactor system. Utilizing automated pumps, the flow of the primary and secondary fluid streams can be regulated and their flow proportioned as desired.

A bioreactor system 500 shown in FIG. 12 utilizes swivel 400 to simultaneously add outflow from the first culture chamber 501 and a second source of media with nutrients and other factors needed by the cells in the second culture chamber 546. The bag assembly 501 constituting the first chamber of the serially arranged culture chambers of the bioreactor system 500 includes bag 520 which has two similar end pieces 505a and 505b for establishing fluid interconnections and positioning the bag assembly 501.

The bag assembly 501 of the system 500 is configured to mount multiple membrane carrier assemblies 27 in an array symmetrical about the longitudinal axis of bag 520. Bag assembly 501 also includes multiple service ports such as a gas venting port 44 and a fill port 40. The fill port 40 is bonded into a penetration in the side of bag 520. The sampling port 44 is also bonded into a penetration in the side of bag 520. The attachments of ports 44 and 40 into bag 520 are reinforced by sealing grommets on both sides of the bag wall, as is well understood by those skilled in the art.

The bag assembly 501 has its constituent components welded or bonded or fused together. Furthermore, all of the components of the bag assembly 501 contacting the biological media are biologically non-reactive, non-toxic and exhibit low protein binding properties.

Preferably the bag 520 is made of a plurality of layers such as the multilayered fabric construction used in the synthesis of custom bags manufactured by Newport Biosystems, Inc. (Anderson, Calif.). Outlet side bag end 505b is a right circular cylindrical disk having a concentric connecting neck 506 on its outer transverse face. Connecting neck 506 has, in sequential order from the outer face of bag end 505b,

26

a straight cylindrical shank, an externally projecting frustro-conical latching shoulder, and a second straight cylindrical outer shank. The inward end of the frustro-conical latching shoulder is a transverse shoulder, while the outward end has the same diameter as that of the second shank.

Coaxial hole 511 is located on the axis of connecting neck 506 and penetrates through the length of connecting neck 506 and part way into the main cylindrical body of bag end 505b. Hole 511 intersects short coaxial cylindrical cavity 512 that has a diameter of approximately 90 percent of the outer cylindrical face of bag end 505b. Flanged disk 507 has a thin outer flange which has the same outer diameter as bag end 505b and a main cylindrical body which is a close fit to the bore of cavity 512 and is bonded thereto, thereby closing cavity 512. The outer side of disk 507 that is exposed to the interior of bag assembly 501 has an array of multiple regularly spaced flat bottom counterbores 514. The array of counterbores 514 may be placed in a hexagonal pattern or in multiple hole circles and may include a counterbore on the axis of bag end 505b, as shown in FIG. 12.

Each counterbore 514 is intercepted by its short coaxial hole 513 that penetrates into cavity 512. Accordingly, a fluid path is created from the hole 511 traversing connecting neck 506, through the cavity 512 and the short connecting holes 513 into each of the counterbores 514. The diameter of each of the counterbores 514 is such that they provide a close fit to the outer diameter of their mated support cylinders 28 of the membrane carrier assemblies 27. Outlet bag end 505b is circumferentially welded or otherwise fused at 508 on its outer cylindrical face to the corresponding end of bag 520 at its end opening. Both the bag ends 505a,b are aligned so that their counterbores 514 are coaxial prior to attachment to the bag 520.

Bag end 505a is substantially identical to bag end 505b, except that instead of having a connecting neck 506, a short axially positioned flat bottom counterbore is provided in the outer end of bag end 505a and swivel 400 is bonded therein, as shown in FIG. 12. This counterbore intersects hole 511, so that an inlet flow path is thereby established.

The membrane carrier assembly 27 transversing the bag assembly 501 has been previously described and is shown in detail in FIG. 3. Each of the carrier assemblies 27 has its ends slipped into and bottomed in the mating counterbores 514 on the interior faces of the bag ends 505a,b. The O-rings 87 provide a seal between the counterbore 514 and the support cylinder 28 of the carrier assembly 27. The membrane 37 is retained in place over the outer diameter of the central portion of support cylinder 28 by the use of O-rings 87 to seal and anchor each end of membrane 37 into each groove 31.

Bag assembly 501 has inlet fluid coupling swivel joint 400, shown in detail in FIG. 11, to allow the bag assembly 501 to freely rotate along with its bag support housing 530 while connected to the primary input fluid feed tubing (not shown) which is attached to hose barb 402. Swivel joint 400 is also attached to a secondary fluid input stream supply tube (not shown) that is connected to hose barb 451 of the swivel. The core piece 440 of swivel 400 is axially mounted in a counterdrilled flat bottom hole on the exterior side of bag end 505a.

Although the bag support housing 530 may be made any shape, the bag support housing is preferably a circular cylinder having transverse end bulkheads 538a,b to ease its horizontal rotation. Bag support housing assembly 530, as described above for housing 930, has the external shape of a right circular cylinder and is hollow so that it has a uniform wall thickness. Bag support housing 530 is provided with

two internal diaphragms **536a,b** that serve to support the outlet and inlet ends of the first culture chamber **501** and second culture chamber **546** respectively. An axial gap is provided between the internal diaphragms **536a,b** of bag support housing **530** for providing space for the interconnections between the first and second bioreactor chamber bags.

Bag support housing **530** has a diametrical split so that it is divided into a top portion **531** and a bottom portion **532**, where the top and bottom portions are hinged together on one cylindrical side of the diametrical split and a latch is provided on the opposed side of the diametrical split. The hinge allows the top portion **531** and the bottom portion **532** of the bag support assembly **530** to be selectably opened and closed, although these features are not explicitly shown in FIG. 12.

Central concentric bores **533a,b,c,d** are located in both the transverse inlet and outlet bulkhead ends **538a,b** and central diaphragms **536a,b** of the bag support assembly **530** and are designed to fit in the external annular grooves around the bag ends **505a,b** and the bag ends **550** and **580** for the second bioreactor chamber bag **546**, while additional offset bores are located on the diametrical split so that they can readily accommodate insertion of fill ports into the split bore. One or more additional radial ports with clearance to permit insertion of a corresponding number of gas removal ports are appropriately positioned in the cylindrical walls of the upper **531** and lower **532** portions of the bag support housing **530**. Although gas removal ports and fill ports are not shown in the cross-sectional view of the second culture chamber **546** shown in FIG. 12, bag **547** has both fill ports and gas removal ports that must be accounted for when designing the bag support housing **530**. The positioning of the radial ports need not be on the diametrical split of the bag support assembly **530**.

A pair of coaxial holes **537** are located parallel to the axis of housing **530** and offset from the axis of the housing so that they are tangent to the inner wall of the housing. These holes **537** serve to accommodate secondary fluid delivery hose **461**, which is attached to hose barb **451** and bypasses the first bioreactor chamber in bag assembly **501** so that the secondary fluid can be delivered to the second bioreactor chamber bag. Bag assembly **501** presses secondary fluid delivery hose **461** against the interior wall of housing **530** when the bag is filled, but the hose **461** is not collapsed. Additionally, annular external tires **540** having cylindrical outer surfaces are provided on the exterior of bag support housing **530** to facilitate driving the housing by means of an external rotational drive system (not shown).

One advantage of bag support housing **530** is that a bag assembly such as **501** made with an inlet and outlet system incorporated into the bag assembly **501** can be inserted into the bag support housing **530** without having to disconnect any tubing or connectors. The bag support housing **530** may be constructed of a variety of materials known in the art, but will preferably be constructed of either a metal, such as stainless steel, or a solid plastic, such as Plexiglas™ or acrylic. A drive assembly rotates the bag support assembly **530** so that the axis of the bag support assembly and its contained bags is horizontal.

Second culture chamber **546** also utilizes a disposable flexible bag **547**, mounted within the support housing **530** between its outlet end bulkhead **538b** and its second internal diaphragm **536b**. The material of the outer wall of bag **547** is similar or identical to that of bag **520**. The bag ends **550** and **580** are supported by central bores **533c** and **533d** in the internal diaphragm **536b** and the bulkhead **538b**, respec-

tively. During fabrication of the second bag, the ends of the bag **547** are sealingly fused to the external cylindrical surfaces of the inlet **550** and outlet **580** bag ends by welds **590**.

To assist in mixing the fluid in the bag assembly **546**, one or more optional perfusion tubes are incorporated into the bag assembly. For example, two types of perfusion tubes **548** and **549** are shown in FIG. 12. These perfusion tubes are used for the purpose of introducing nutrients into the interior volume of second bioreactor chamber **545**. In the cross-sectional view of FIG. 12, two attached outer perfusion tubes **548** are mounted on the interior of the bag wall. Each outer perfusion tube **548** is supported over its length, beginning a small distance from the inlet plenum **551** of inlet bag end **550**, by being joined integrally with a lapped bag seam. The perfusion tube **548** is symmetrically positioned in the seam and fused to the wall of bag **547** by welds on each side of seam. The weld joints only cover a portion of the circumference of the outer perfusion tubes **548**, so that a substantial portion of the tubes is still exposed to the interior volume of the bag **547**.

The bag **547** may also have one or more cantilevered internal perfusion tubes **549** of various configurations as seen in FIGS. 9, 10 and 12. For example the perfusion tubes **549**, shown in FIG. 12, extend from the inlet transverse end cap **558** into the interior space **545** of the bag **547**. The internal perfusion tubes **548** and **549** will vary in length, weight and flexibility, depending on the viscosity of the media. The internal perfusion tubes **548** and **549** move with the rotation of the bag support **530**, thus adding to the mixing of the media within the bag.

Each perfusion tube **548** and **549** has a distal closure **560** where the perfusion tube is closed or sealed by crimping and/or fusing. In contrast, the inlet end of each perfusion tube **548** and **549** is in communication with the fluid inlet plenum **551** for the bag assembly **546**. The material of construction of perfusion tubes **548** and **549** is a biologically-compatible, non-toxic, non-protein-absorbing, flexible polymer having a number of perforations or a controlled permeability for the nutrient fluids for the biological media of the second chamber **545**, allowing fresh media to be pumped into the bag assembly **546** under a low pressure gradient, similar to a soaker hose used in watering lawns.

The exterior of the body of inlet bag end **550** is a relatively thin walled right circular cylinder that is sized to have a slip fit within the central bore of the bag support closure **530** or within the central bore of the hinged bag support assembly. On the interior end of inlet bag end **550** is the integral thin disk inlet end cap **558**, while on the exterior end of inlet bag end **550** is thin integral planar annular ring **559**. Inlet end cap **558**, annular ring **559**, and the interior of the cylinder of inlet bag end **550** form inlet plenum **551**. Concentrically attached to the exterior side of annular ring **559** of inlet bag end **550** is cylindrical inlet neck **552**. The through bore **553** of inlet neck contains, in order from its external end, an annular latching groove detent engagable with the latching shoulder of connecting neck **506** of the first chamber and a female O-ring groove mounting an O-ring. The bore **553** is a close fit to the cylindrical exterior of connecting neck **506**, thereby permitting the O-ring in the cylindrical inlet neck **552** to seal to connecting neck **506**.

Radial secondary inlet hose barb **554** has concentric interior through barb bore **555** that penetrates to the bore **553** in the interior of inlet neck **552**. The downstream end of secondary flow tube **461** is attached to inlet hose barb **554**, thereby permitting comingling of the outlet flow stream from the first chamber **501** and the secondary nutrient flow

for the second chamber **546**. The commingled flow stream freely passes to the inlet plenum **551** for distribution into the interior **545** of the bag **547** through the tubes **548** and **549**.

Multiple radial branch ports extend through the cylindrical wall of inlet bag end **550** and intersect inlet plenum **551**. An attached outer perfusion tube **548** is fused into each branch port so that the bore of each tube **548** is in communication with inlet plenum **551**. Likewise, multiple interior perfusion tubes **549** are fused into corresponding branch ports that are provided in inlet end cap **558** coplanar with and at an angle ranging from 0° to 45° to the axis of the inlet end cap. Each cantilevered interior perfusion tube **549** thus has its bore in communication with inlet plenum **551**.

Outlet bag end **580** is a right circular cylinder that has a coaxial flat bottom counterbore **581** in its interior transverse end and a connecting neck **582** for a fluid swivel **50** (not shown) that conveys the outflow from the second chamber **546** to storage or processing. Connecting neck functionally is substantially similar to connecting neck **506** of the first chamber **501**. Concentric through bore **583** passes through neck **582** and intersects counterbore **581**.

Controlled porosity cylindrical filter disk **586**, which has a thickness less than the depth of counterbore **581**, is sealingly mounted externally flush in counterbore **581**, thereby creating collector plenum **587** between the filter disk **586** and the counterbore **581**. Outflow fluid emerging from the second chamber **546** is filtered by disk **586** to prevent the cells within the second chamber from leaving that chamber, while at the same time waste products and the desired output products produced within second chamber **546** are passed by filter disk **586**.

EXAMPLE 9

Interconnected Disposable Culture Chambers

FIG. 13 shows a bioreactor system **600** having serially arranged biological culture chambers **602** and **645**. The bioreactor system **600** is similar in several ways to the bioreactor system **500** and uses several common components. However, rather than using a second chamber bag with the perfusion tubes disclosed in FIG. 10, the bioreactor system **600** uses bags with multiple interior membrane carrier assemblies **27** or components substantially similar to the membrane carrier assemblies **27** for both the first chamber **602** and the second chamber **645**.

Referring to FIG. 13, the bag assembly **501** constituting the first chamber **602** includes bag **520** that has two similar end pieces **505a** and **505b** for establishing fluid interconnections and positioning the bag assembly **501**. The bag assembly **501** of the system **600** is configured to mount multiple membrane carrier assemblies **27** in an array symmetrical about the longitudinal axis of bag **520**. Bag assembly **501** includes multiple service ports such as a gas venting port **44** and a fill port **40**. The bag assembly **501** has its constituent components welded or bonded or fused together. Furthermore, all of the components of the bag assembly **501** contacting the biological media are biologically non-reactive, non-toxic and exhibit low protein binding properties.

Outlet side bag end **505b** is a right circular cylindrical disk having a concentric connecting neck **506** on its outer transverse face. Flanged disk **507** has a thin outer flange which has the same outer diameter as bag end **505b** and a main cylindrical body which is a close fit to the bore of cavity **512** and is bonded thereto, thereby closing cavity **512**. The outer side of disk **507** that is exposed to the interior of bag assembly **501** has an array of multiple regularly spaced flat

bottom counterbores **514**. The array of counterbores **514** may be placed in a hexagonal pattern or in multiple hole circles and may include a counterbore on the axis of bag end **505b**, as shown in FIG. 13. Each counterbore **514** is intercepted by its short coaxial hole **513** that penetrates into cavity **512**. Outlet bag end **505b** is circumferentially welded or otherwise fused at **508** on its outer cylindrical face to the corresponding end of bag **520** at its end opening. Both the bag ends **505a,b** are aligned so that their counterbores **514** are coaxial prior to attachment to the bag **520**.

Bag end **505a** is substantially identical to bag end **505b**, except that instead of having a connecting neck **506**, a short axially positioned flat bottom counterbore is provided in the outer end of bag end **505a** and swivel **400** is bonded therein, as shown in FIG. 13. This counterbore intersects hole **511**, so that an inlet flow path is thereby established.

The membrane carrier assembly **27** transversing the bag assembly **501** has been previously described and is shown in detail in FIG. 3. Each of the carrier assemblies **27** has its ends slipped into and bottomed in the mating counterbores **514** on the interior faces of the bag ends **505a,b**. Bag assembly **501** has inlet fluid coupling swivel joint **400**, shown in detail in FIG. 9, to allow the bag assembly **501** to freely rotate along with its bag support housing **530** while connected to the primary input fluid feed tubing (not shown) which is attached to hose barb **402**. Swivel joint **400** is also attached to a secondary fluid input stream supply tube (not shown) that is connected to hose barb **451** of the swivel. The core piece **440** of swivel **400** is axially mounted in a counterdrilled flat bottom hole on the exterior side of bag end **505a**.

The bag support housing **530** is basically the same unit as used for the bioreactor system **500** and described above. The bag support housing **530** is a circular cylinder having transverse end bulkheads **538a,b** and provided with two internal diaphragms **536a,b** that serve to support the outlet and inlet ends of the first **602** and second **645** culture chambers, respectively. An axial gap is provided between the internal diaphragms **536a,b** of bag support housing **530** for providing space for the interconnections between the first and second culture chamber bags.

Bag support housing **530** has a diametrical split so that it is divided into a top portion and a bottom portion, where the top and bottom portions are hinged together on one cylindrical side of the diametrical split and a latch is provided on the opposed side of the diametrical split. The hinge allows the top portion and the bottom portion of the bag support assembly **530** to be selectably opened and closed. Central concentric bores **533a,b,c,d** are located in both the transverse inlet and outlet bulkhead ends **538a,b** and central diaphragms **536a,b** of the bag support assembly **530** and are designed to fit in the external annular grooves around the bag ends **505a,b** and the bag ends **505c,d** for the second culture chamber bag **645**, while additional offset bores are located on the diametrical split so that they can readily accommodate insertion of fill ports into the split bore. One or more additional radial ports with clearance to permit insertion of a corresponding number of gas removal ports are appropriately positioned in the cylindrical walls of the upper and lower portions of the bag support housing **530**.

A pair of coaxial holes **537** are located parallel to the axis of housing **530** and offset from the axis of the housing so that they are tangent to the inner wall of the housing. These holes **537** serve to accommodate secondary fluid delivery hose **461**, which is attached to hose barb **451** and bypasses the first bioreactor chamber in bag assembly **501** so that the secondary fluid can be delivered to the second bioreactor

31

chamber bag assembly **645**. Bag assembly **501** presses secondary fluid delivery hose **461** against the interior wall of housing **530** when the bag is filled, but the hose **461** is not collapsed. Additionally, annular external tires **540** having cylindrical outer surfaces are provided on the exterior of bag support housing **530** to facilitate driving the housing by means of the external rotational drive system (not shown). The drive assembly rotates the bag support assembly **530** so that the axis of the bag support assembly and its contained bags is horizontal.

The second bioreactor chamber **645** also utilizes a disposable flexible bag assembly identical to that of bag assembly **501** except for two modifications. The first modification is that the bag **675** may have a different volume than the first bag assembly **501**. This may be accomplished by making the bag **645** longer and fitting it with longer membrane carrier assemblies **627** that mount different molecular cutoff membranes **637**. The second modification to second chamber **645** is that the inlet swivel **400** of the first bag assembly **501** is replaced by an inlet neck **552** which is suitable for connection to both outlet connecting neck **506** of bag assembly **501** and to the secondary supply tube **461**. In order to mount inlet neck **552** for second chamber **645**, the upstream bag end piece **505c** is made identically to bag end piece **505a** of bag assembly **501** except that the coaxial counterbore on the exterior flat face is made larger.

The second bioreactor chamber **645** is also mounted coaxially within the support housing **530** between its outlet end bulkhead **538b** and its second internal diaphragm **536b**. The bag ends **505c,b** of the second chamber **645** are supported by central bores **533c** and **533d** in the internal diaphragm **536b** and the bulkhead **538b**, respectively.

Concentrically sealingly attached to the exterior side of inlet bag end **505c** of second chamber **645** is cylindrical inlet neck **552**. The through bore **553** of inlet neck contains, in order from its external end, an annular latching groove detent engagable with the latching shoulder of connecting neck **506** of first bag assembly **501** and a female O-ring groove mounting an O-ring. The bore **553** is a close fit to the cylindrical exterior of connecting neck **506**, thereby permitting the O-ring in the cylindrical inlet neck **552** to seal to connecting neck **506**.

Radial secondary inlet hose barb **554** has concentric interior through barb bore **555** that penetrates to the bore **553** in the interior of inlet neck **552**. The downstream end of secondary flow tube **461** is attached to inlet hose barb **554**, thereby permitting comingling of the outlet flow stream from the interior of the first chamber **502** and the secondary nutrient flow for the second chamber **645**. The comingling flow stream freely passes to the carrier assemblies **627** for distribution into the interior of bag assembly **645**.

The longer membrane carrier assemblies **627** are identical, except for their length and selection of the molecular cutoff membrane **637**, to the previously described membrane carrier assemblies **27** used in the first bag assembly **501** of this embodiment and described in the disclosure of the bioreactor system **10** of the present invention. The support cylinder is made longer in its central section to support a longer membrane **637**, but otherwise does not differ from the construction of cylinder **28**. The material of membrane **637** may be different from that of membrane **37**, in that a different molecular cut-off value may be required in order to obtain optimal disposition of the biological media and end products from the second culture chamber **645**.

The operation of the first **501** and second **645** bioreactor chambers is similar to that described previously, in that the first chamber **501** contains cells supplied with the primary

32

nutrient stream through the inlet hose barb **402** of swivel **400** and via the membrane carrier assemblies **27** mounted within chamber **501**. Nutrients diffuse outwardly through the membranes **37** of carrier assemblies **27** and waste products and the desired growth hormones, precursors, stimulants, or moderator compounds diffuse inwardly through membranes **37**. The outflow stream from first chamber **501** is then supplied to the inlet neck **552** of the second chamber **645** where it is cominglinged with the bypassed secondary nutrient stream from tube **461**. The cominglinged stream is then supplied to the membrane carrier assemblies **627** of the second chamber **645** where its nutrients perfuse outwardly through membranes **637**. The waste products from the second chamber, along with the desired products perfuse inwardly through membranes **637** and then flow out of the bioreactor system **600** through connecting neck **506** of second chamber **645**, through a swivel **50** (not shown), and thence into storage or processing.

Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. A culture system comprising:

- (a) a fluid inlet;
- (b) a first culture compartment having a tubular housing;
- (c) a first end piece attached to the fluid inlet on one side and to a first end of the tubular housing on a second side;
- (d) a second culture compartment coaxial with the first culture compartment, the second culture compartment having a proximal end and a distal end;
- (e) a fluid connector having a first side mounted on a second end of the tubular housing and a second side mounted on the proximal end of the second culture compartment, the fluid connector having a through bore passing from the first side to the second side of the fluid connector, wherein the through bore directs a fluid stream from the first culture compartment into the second culture compartment;
- (f) a connector filter positioned on the first side of the fluid connector to filter a fluid stream passing out of the first culture compartment and into the through bore of the fluid connector;
- (g) a fluid outlet;
- (h) a distal end piece mounted on the distal end of the second culture compartment and connected to the fluid outlet; and
- (i) an outlet filter transversing the second culture compartment including:
 - a support cylinder having a first end supported by the fluid connector and a second end supported by the distal end piece,
 - a molecular weight cut-off membrane secured to an exterior surface of the support cylinder, and
 - a chamber between the exterior surface of the cylinder and an interior surface of the membrane, the chamber in fluid communication with the through bore of the fluid connector and the fluid outlet.

2. A culture system comprising:

- (a) a fluid inlet;
- (b) a first culture compartment having a tubular housing made of a fluid-impenetrable material, wherein the tubular housing has a first end and an opposed second end;

33

- (c) a first end piece attached to the fluid inlet on one side and to the first end of the tubular housing on a second side,
 - (d) a second culture compartment coaxial with the first culture compartment and in fluid communication with the first culture compartment, the second culture compartment having a proximal end and a distal end;
 - (e) a fluid connector having a first side mounted on the second end of the tubular housing and a second side mounted on the proximal end of the second culture compartment, the fluid connector having a through bore passing from the first side to the second side of the fluid connector;
 - (f) a connector filter having a first end and a second end, wherein the first end is mounted on the first side of the fluid connector, the connector filter positioned to filter a fluid stream passing out of the first culture compartment into the through bore of the fluid connector and into the second culture compartment;
 - (g) a fluid outlet;
 - (h) a distal end piece mounted on the distal end of the second culture compartment and connected to the fluid outlet; and
 - (i) an outlet filter having a one end mounted on a proximal side of the distal end piece, wherein the outlet filter is a membrane carrier assembly transversing the second culture compartment wherein the membrane carrier assembly has:
 - a support cylinder;
 - a molecular weight cut-off membrane secured to an exterior surface of the support cylinder, and
 - a chamber between the exterior surface of the cylinder and an interior surface of the membrane, the chamber in fluid communication with the through bore of the fluid connector and the fluid outlet.
3. The culture system of claim 2, wherein the through bore of the fluid connector is intersected by a through bore of a second fluid inlet.
4. The culture system of claim 2, further comprising at least one penetration port extending through a wall of the first or second culture compartment.
5. The culture system of claim 2, further comprising a gas venting means for allowing gas to escape from the first or second culture compartment as the compartment is filled with fluid.
6. The culture system of claim 2, further comprising a fill means for inserting fluids into or removing fluids out of the first or second culture compartment.
7. The culture system of claim 2, wherein the first end, the distal end and the fluid connector are concurrently rotated by a drive assembly.
8. The culture system of claim 2, wherein the second culture compartment has a greater volume than the first culture compartment.
9. The culture system of claim 2, further comprising an identifier.
10. The culture system of claim 9, wherein the identifier is a bar code.
11. A culture system comprising:
- (a) a fluid inlet;
 - (b) a first culture compartment having
 - (i) a fluid-impenetrable tubular sleeve having a first end and an opposed second end,

34

- (ii) a growth compartment within the sleeve, and
 - (iii) a first end piece having one side attached to the fluid inlet and a second side attached to a first end of the tubular sleeve;
 - (c) a second culture compartment coaxial with the first culture compartment, the second culture compartment having
 - (i) a fluid-impenetrable housing having a proximal end and a distal end; and
 - (ii) a growth compartment within the housing that is in fluid communication with the growth compartment within the sleeve,
 - (d) a fluid connector having a first side mounted on the second end of the tubular sleeve and a second side mounted on the proximal end of the housing, the fluid connector having a through bore passing from the first side to the second side of the fluid connector wherein the through bore is in fluid communication with the growth compartment of the first and second culture compartment;
 - (e) a connector filter having a one end supported by the first side of the fluid connector;
 - (f) a membrane carrier assembly transversing the second culture compartment comprising
 - (i) a support cylinder,
 - (ii) a molecular weight cut-off membrane secured to an exterior surface of the support cylinder, and
 - (iii) a chamber between the exterior surface of the cylinder and an interior surface of the membrane, the chamber in fluid communication with the through bore of the fluid connector and the growth compartment within the housing;
 - (g) a fluid outlet; and
 - (h) a distal end piece mounted on the distal end of the second culture compartment and connected to the fluid outlet.
12. The culture system of claim 11, wherein the connector filter includes a molecular weight cut-off membrane.
13. The culture system of claim 12, wherein the connector filter includes a molecular weight cut-off membrane having a different molecular weight cut-off than the molecular weight cut-off membrane of the membrane carrier assembly.
14. The culture system of claim 12, wherein the molecular weight cut-off membrane of the connector filter is identical to the molecular weight cut-off membrane of the membrane carrier assembly.
15. The culture system of claim 11, wherein the connector filter includes:
- a cylindrical support transversing the first culture compartment, the support having a first end supported by the first end of the sleeve and a second end supported by the first side of the fluid connector;
 - a molecular weight cut-off membrane secured to an exterior surface of the cylindrical support, and
 - a channel between the exterior surface of the cylindrical support and an interior surface of the membrane, the channel in fluid communication with the through bore of the fluid connector and the growth compartment of the first and second culture compartment.

* * * * *